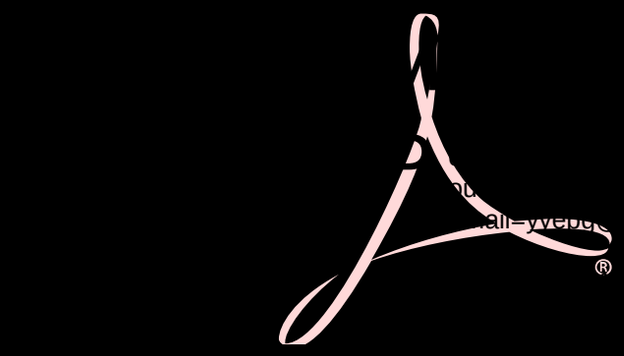

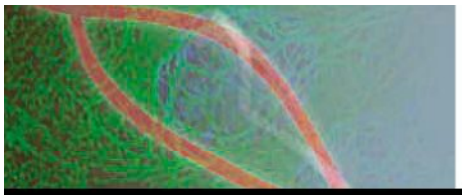

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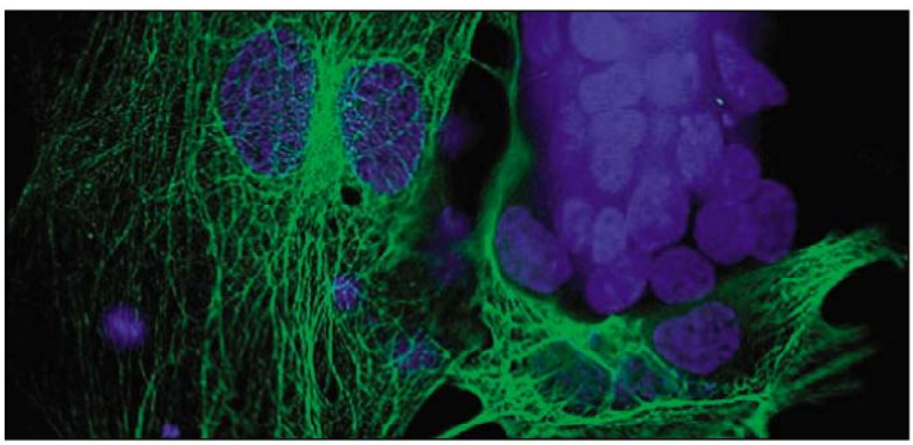

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1. Sharma *et al.* (2005) *Cancer Res.* **65(6):** 2412.

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Science

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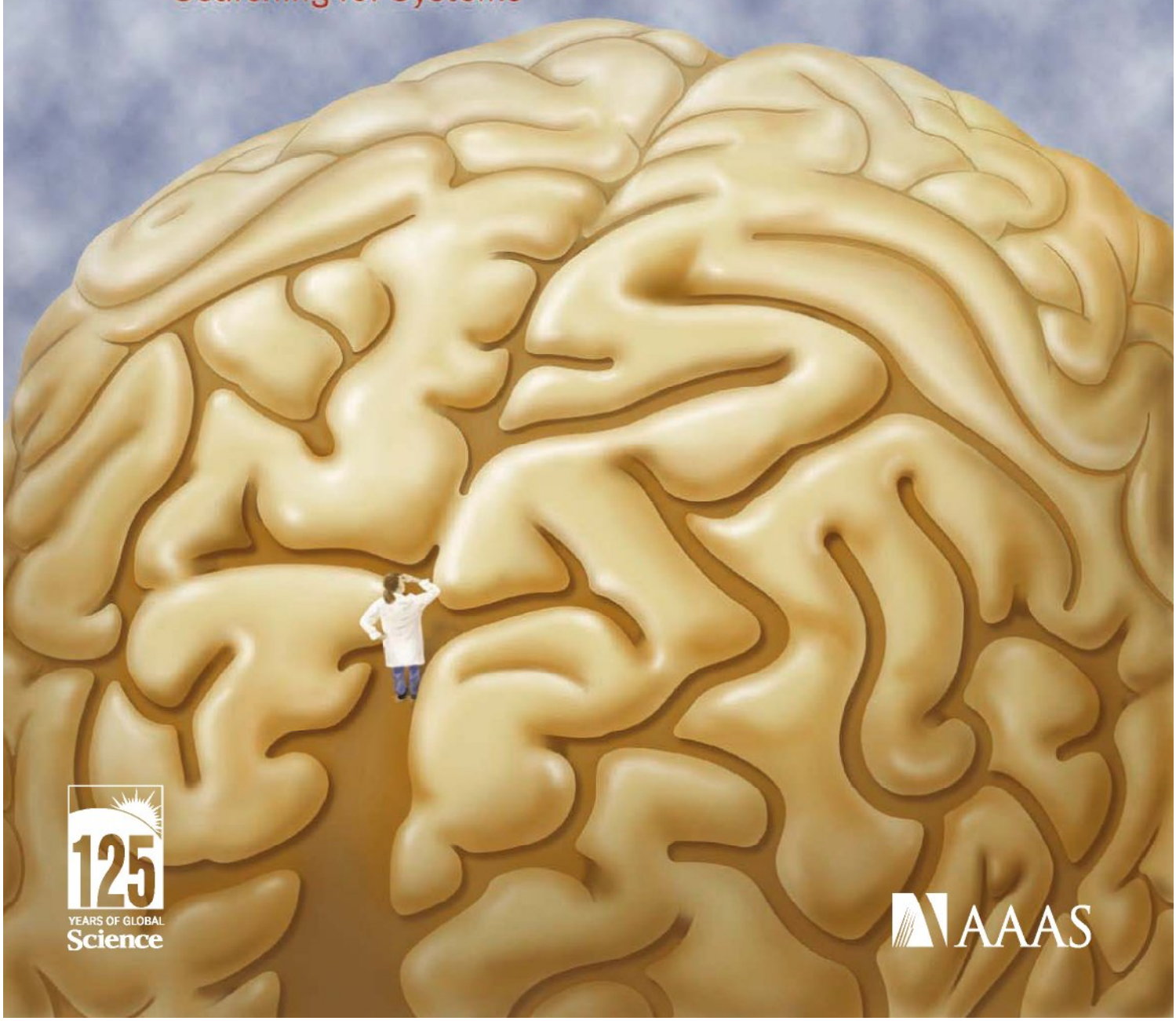

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SYSTEMS-LEVEL BRAIN DEVELOPMENT

Volume 310
4 November 2005
Number 5749

A special section examines how a systems-level analysis of brain development links cell biology to psychiatry. Researchers are making their way past the surface complexities of the brain to begin to understand the influences and pathways that establish thought, action, and personality. [Image: Katharine Sutliff]

INTRODUCTION

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VIEWPOINTS

Related Editorial page 745; Science Express Report by M. Tassabehji et al.

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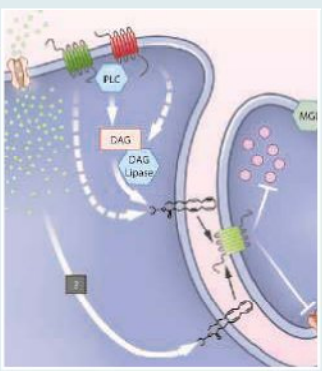
EDITORIAL GUIDE: **Focus Issue—Memories of Signals Past: Plastic Changes in Nervous System Function** *E. M. Adler and N. R. Gough*
Molecular changes underlie synaptic plasticity in response to physiological or pathological stimuli.

PERSPECTIVE: **Endocannabinoid Identification in the Brain—Studies of Breakdown Lead to Breakthrough, and There May Be NO Hope** *B. E. Alger*
How do endocannabinoids mediate depolarization-induced suppression of inhibition?



PERSPECTIVE: **Hijacking the ERK Signaling Pathway—*Mycobacterium leprae* Shuns MEK to Drive the Proliferation of Infected Schwann Cells** *L. A. Noon and A. C. Lloyd*
M. leprae subverts the normal mechanisms regulating Schwann cell proliferation.

REVIEW: **The "Ups and Downs" of Signaling Cascades in Addiction** *D. Ron and R. Jurd*
Elucidation of signaling cascades involved in addiction may lead to new therapeutic approaches.

TEACHING RESOURCE: **Long-Term Potentiation—Mechanisms of Induction and Maintenance** *R. D. Blitzer*
Prepare a graduate-level class about the signals responsible for LTP induction and maintenance.

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GLOBAL: **Careers in Neuroscience Research—Feature Index** *A. Forde*
Next Wave profiles neuroscientists whose passion and dedication has led to outstanding research.

GLOBAL/US: **Crossroads in Neuroscience** *J. Kling*
Two young scientists are studying brain imaging and behavior and the molecular cues that guide neuronal growth.

GLOBAL/CANADA: **Getting Wired—Pathway of a Neuroscientist** *A. Fazekas*
Edward Ruthazer of McGill University's Montreal Neurological Institute talks about his fulfilling career.

GLOBAL/EUROPE: **Neurology in the Lab, and at Patients' Bedsides** *E. Pain*
Neurology was Diego Centonze's second choice, but it opened up a whole new world of research.

GLOBAL/MISCINET: **Investigating the Neural and Vascular Consequences of Stroke** *R. Arnette*
Byron Ford has a varied background that enabled him to carve out a unique niche in stroke research.

GLOBAL/GRANTSNET: **Funding Opportunities in Neuroscience** *Edited by S. Martin*
GrantsNet offers a sampling of funding opportunities for neuroscience research.

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PERSPECTIVE: **Keeping Priorities—The Role of Working Memory and Selective Attention in Cognitive Aging** *J. W. de Fockert*
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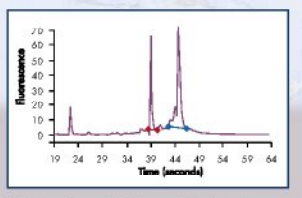

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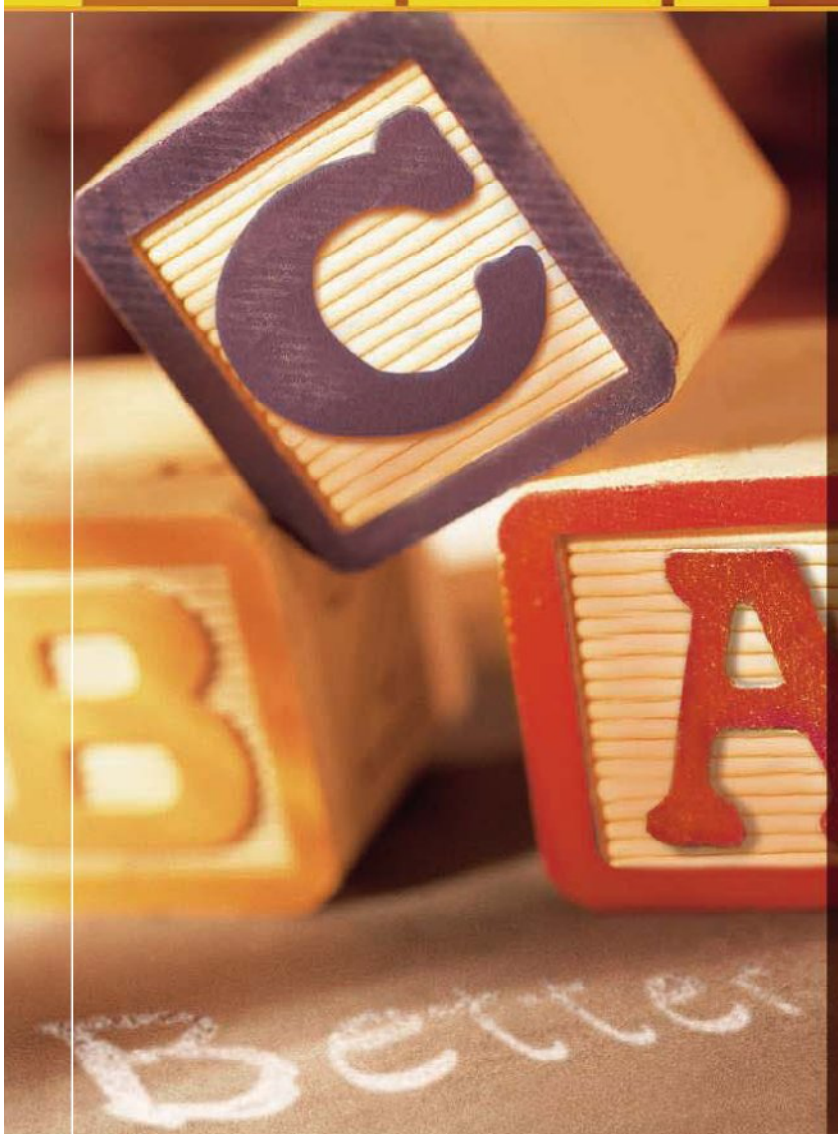

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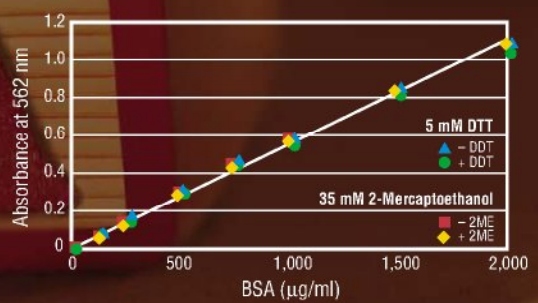

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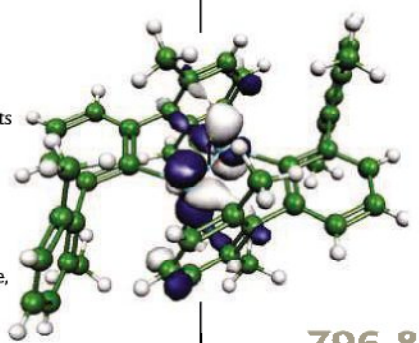

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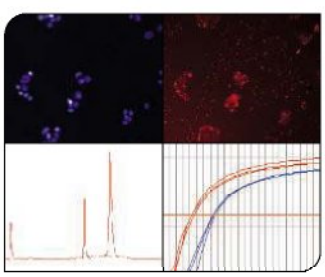

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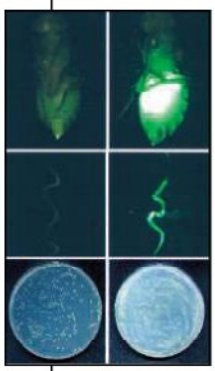

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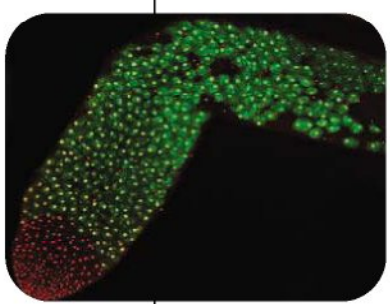

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PERSPECTIVE: Developing a Research Agenda in Biogerontology—Basic Mechanisms *H. R. Warner*
The author describes how research initiatives have been developed at the National Institute on Aging over the past two decades.

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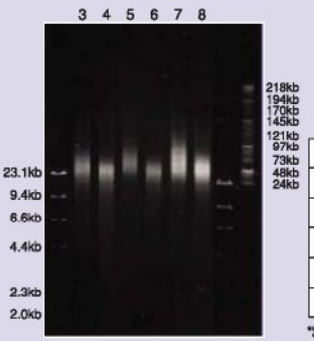

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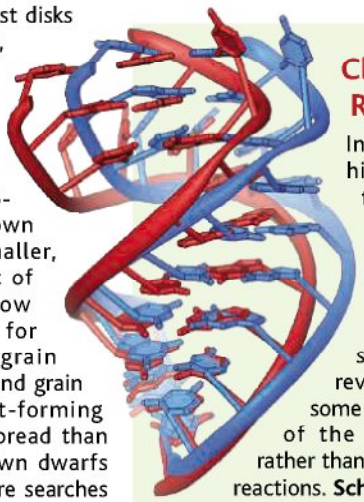
More Prolific Planet Production

As the center of a collapsing dust cloud heats up and approaches the conditions for star formation, the dust grains settle into a plane to form a disk around the central core. The particles in this disk eventually combine to form planets and asteroids. Although this picture is consistent with observational data for the brighter dust disks of intermediate mass stars, little is known about planet formation around smaller stars. **Apai *et al.*** (p. 834, published online 20 October 2005) present infrared spectroscopic observations of protoplanetary disks around brown dwarfs, objects that are smaller, cooler, and often just short of being stars. The spectra show signs of three key markers for planet formation—dust grain growth, grain crystallization, and grain settling. This kind of planet-forming process may be more widespread than once thought, and thus brown dwarfs should be candidates for future searches for planets outside our solar system.



Close Up of the Ribosome

In the last few years, high-resolution structures of the 30*S* and 50*S* bacterial ribosome subunits have revealed significant insights into the mechanism of protein synthesis, in particular revealing that the ribosome is a ribozyme—some of the constituent RNAs, rather than proteins, catalyze key reactions. **Schuwirth *et al.*** (p. 827; see the Perspective by **Moore**) describe two structures of the intact *Escherichia coli* ribosome at 3.5 angstrom resolution. The structures show details of the interaction interface and peptidyl transferase center and reveal molecular motions that are likely to be involved in messenger RNA and transfer RNA translocation.

A Wetter Upper Troposphere

If increased levels of carbon dioxide (CO_2) were the only cause of global warming, we could expect the worldwide average surface temperature to increase by about 1°C during this century. However, climate models project warming roughly three times that much because of feedback effects from water vapor. Up until now, experimental evidence that upper tropospheric water vapor content has actually been increasing has been lacking. **Soden *et al.*** (p. 841, published online 6 October 2005; see the Perspective by **Cess**) have used satellite observations to reveal a distinct radiative signature of increasing upper tropospheric moisture from 1982 to 2004. This moistening is consistent with model reconstructions for the same period.

A Quintuple Bond

Covalent bonding, or electron sharing between atoms, is the basis of molecular chemistry. In principle, two transition metal atoms can share up to 12 electrons before repulsion starts to push them apart, rather than keeping them together. In practice, however, the quadruple bond of eight shared electrons has been the highest stable interaction in isolated compounds; beyond this number, clusters tend to form instead. **Nguyen *et al.*** (p. 844, published online 22 September 2005; see the Perspective by **Frenking**) have used bulky triphenyl ligands to stabilize a chromium dimer in which one more pair of electrons is shared. Although x-ray crystallography reveals a bent geometry, theory and magnetic measurements support participation of all of the metal d orbitals in the bond.

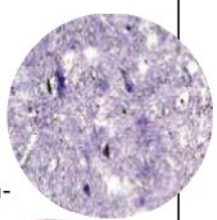
Intermittent Ionosphere Layer

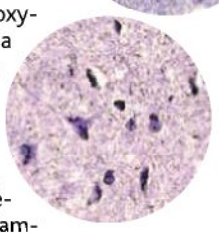
Mars's ionosphere, which extends from about 110 to 135 kilometers (km) above the planet, consists of two distinct layers and helps protect the lower atmosphere from removal by the solar wind. The existence of a third lower layer has been predicted, and as discussed by **Pätzold *et al.*** (p. 837), has now been detected by radio-wave observations by Mars Express. However, this third layer, which is seen to extend to as low as 65 km above the planet, appears to be intermittent, not permanent as was expected, and is likely formed from the ablation of meteorites.

Decay Discrepancy Reconciled

The beta decay of ^{176}Lu to ^{176}Hf is an important isotopic system for tracing the geochemical evolution of Earth and other planets, as these elements are fractionated by the formation of continental crust. Application, and particularly comparison to other decay systems requires accurate knowledge of the decay constant (or half-life. However, comparison of the systematics in meteorites and terrestrial rocks have yielded two different values, a discrepancy that has even raised suggestions that other energetic processes might be affecting decay constants. **Amelin** (p. 839) selected specific samples from meteorites that also have accurate uranium-lead dates and shows that the half-life in meteorites is the same as that in other rocks.

Breakdown to Recovery





Regulation of immune responses through local catabolic depletion of tryptophan (Trp) was first identified in studies of the maternal T cell response to the fetus. This pathway, which is controlled by the enzyme indoleamine 2,3-dioxygenase (IDO), has since been identified in a variety of immunological settings. **Platten *et al.*** (p. 850) now find that IDO-mediated Trp catabolism also contributes during therapy of a mouse model of multiple sclerosis. By using a form of antigen, termed an altered peptide ligand, T cell responses were prevented from causing inflammation and nervous system pathology, and this effect corresponded with the induction of IDO. Naturally occurring metabolites and a synthetic derivative of the IDO pathway inhibited T cell proliferation and activation of antigen-presenting cells. Remarkably, paralyzed mice recovered after being fed the synthetic derivative.

CONTINUED ON PAGE 743

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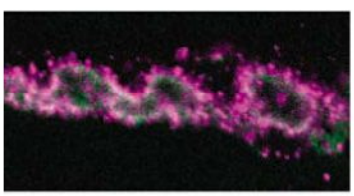
A Gut Oxidase Reaction

The mucosal surfaces of the body are constantly exposed to microorganisms and have evolved a variety of protective innate immune mechanisms, including the generation of antimicrobial peptides throughout the animal kingdom. Another hallmark of the innate immune response is the generation of microbicidal reactive oxygen species (ROS) by phagocytes. **Ha *et al.*** (p. 847) observe that gut mucosal epithelial cells of *Drosophila* expressed dual oxidase (dDuox) upon bacterial infection. Flies in which dDuox expression had been silenced were significantly more susceptible to infection, and protection could be restored upon dDuox reexpression. Similar mucosal ROS production mechanisms may be exploited in host defense across different species.

Gliding Motility Factors on the Move

Little is known about the directional determinants of so-called gliding motility in bacteria. **Mignot *et al.*** (p. 855) now show that FrzS, a protein essential for pilus-based gliding motility in *Myxococcus xanthus*, moves in an oscillatory pattern by disassembling and reassembling clusters at the cell poles as cells reverse their direction of movement. The frequency of the oscillations is controlled by the Frz chemosensory system, which is essential for directed motility. Pole-to-pole migration of FrzS appears to involve directed movement along the length of the cell.



CREDIT: YOSHIHARA *ET AL.*

From Post- to Presynaptic Sites

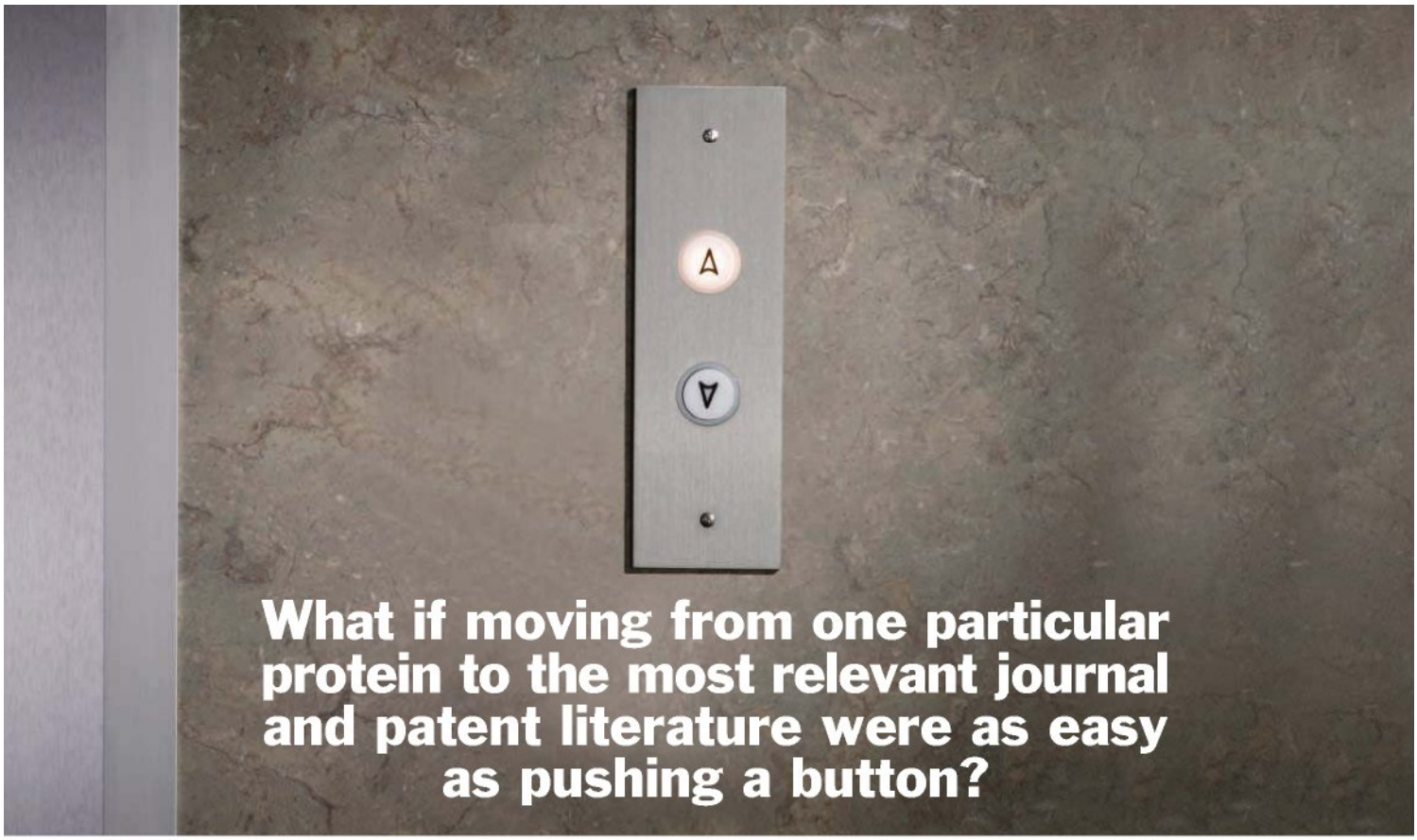
Postsynaptic Ca^{2+} signals are somehow transduced into alterations in presynaptic function during enhanced synaptic activity. **Yoshihara *et al.*** (p. 858) show that in the *Drosophila* embryonic neuromuscular junction, miniature endplate potential induction by presynaptic stimulation is blocked by postsynaptic Ca^{2+} chelation or genetic ablation of synaptotagmin 4 (Syt 4). This blockage can be rescued by postsynaptically targeted "knockin" of Syt 4. Similarly, the reduced amplitude of endplate potentials in Syt 4–deficient mutant can be rescued by postsynaptic rescue of Syt 4. Postsynaptic Syt 4 thus up-regulates transmitter release.

Object Recognition in a Flash

Humans and other primates have the astonishing ability to recognize and categorize objects within 200 milliseconds. By using a classifier-based decoding approach, **Hung *et al.*** (p. 863) characterized the neuronal representation for object recognition in monkey inferotemporal cortex (area IT) and quantitatively examined the underlying neural code. Surprisingly, the activity of small numbers of neurons over very short periods of time was sufficient to support rapid and accurate recognition of object category and identity, which was at the same time invariant to large changes in object position and scale.

Wringing the Neck

The susceptibility of individual synapses to plasticity induction may be influenced by the ability of signaling molecules to move into and out of the head of the dendritic spine. Thus, regulation of protein movement by the spine neck offers a potentially powerful mechanism to control individual synapses. **Bloodgood and Sabatini** (p. 866) found that diffusional equilibration across the necks of dendritic spines is directly regulated by activity. By combining two-photon microscopy with two-photon laser photoactivation, protein movement was measured across the necks of a large population of spines. A subclass of spines was effectively isolated from the dendrite. Spine compartments have traditionally been treated as static entities, but it now appears that spine-dendrite coupling is strongly dynamic. Diffusion barriers vary considerably over time in a way that reflects the cell's recent history of spiking.



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Pedagogy Meets Neuroscience

Having acknowledged the social and economic value of education, modern societies are increasingly making concerted efforts to improve schooling at all age levels. Today, policy-makers and practitioners responsible for educational reform and improving classroom practice seek to base their decisions on empirical evidence rather than on opinions, fashions, and ideologies, as was too often the case in the past. This desire for "evidence-based" education has coincided with a period of tremendous progress in the field of neuroscience and enormous public interest in its findings, leading to an ongoing debate about the potential of neuroscience to inform education reform. Although the value of neuroscience research on this front is seemingly promising, collaboration with educators is doomed to failure if the public is not correctly informed and if the research is not considered in an interdisciplinary context.

It has become dangerously fashionable to label general—even trivial—pedagogical advice that is not grounded in scientific fact as "brain-based learning." For instance, findings about rapid synaptic proliferation in young children's brains have nurtured hopes that cognitive capabilities can be increased by teaching infants vocabularies and basic facts with audiovisual material. But proponents of these early education programs have conveniently overlooked the lack of direct empirical evidence linking neurological and learning processes. It is far from clear whether children who are encouraged to memorize isolated facts early in life show better long-term retention than their peers.

As a scientist specializing in school-related learning, I am open to the educational implications of neuroscience. However, we need to scale down unrealistic expectations. Otherwise, there is a danger that new efforts to incorporate research in this area into education could be stymied by falsely raising the hopes of the public and policy-makers. There is the further danger that people will ignore the importance of empirical research in the fields of educational and instructional science, psychology, and information technology—work that can continue to teach us about good schooling. Thanks to these more traditional areas of research, we understand a great deal about what has gone wrong in learning environments when otherwise competent students fail to learn. Research on learning and instruction has provided precise and applicable knowledge about how to design powerful learning environments in many content areas. What we now know about the conditions under which pictorial representations aid in teaching advanced concepts goes far beyond the recommendations of so-called brain-based learning.

Nevertheless, certain groups of learners do not benefit sufficiently from educational environments developed in accordance with state-of-the-art research on learning and instruction, and here is where collaboration among traditional research disciplines and neuroscience may be promising. Looking into the brain during problem solving might help to clarify what impedes learning. For instance, there is an ongoing debate on whether male students outperform female students in mathematics and science because of their greater ability to use visual-spatial representations as reasoning tools. As yet, however, the implications of achievement data and behavioral observations remain ambiguous in this respect. Neuroimaging techniques have elucidated areas of the brain that are especially involved in visual-spatial processing, so we may be able to find out whether differences in achievement can be traced back to the use of visual-spatial representations in reasoning. Similarly, neuroimaging may help to clarify whether visual or phonological processing is impaired in people with dyslexia.

Neuroscience may also be able to show how prior experiences can improve learning, going beyond psychological explanations. Although many studies have found evidence for the overwhelming impact of prior knowledge of skills, procedures, or concepts on learning, there may be other ways of improving learning besides such knowledge transfer. Cognitive activities can stimulate certain neuronal processes by triggering electrical impulses and the release of neurotransmitters in particular brain areas. Concurrently, other cognitive activities that are processed in similar brain areas may be enhanced, even if the two cognitive activities involve completely different knowledge structures.

Neuroscience alone cannot provide the specific knowledge required to design powerful learning environments in particular school content areas. But by providing insights into the abilities and constraints of the learning brain, neuroscience can help to explain why some learning environments work while others fail. As part of interdisciplinary collaborations, neuroscience is poised to help structure the future classroom. This would be "evidence-based" reform worth supporting.

Elsbeth Stern

Elsbeth Stern is professor of cognitive and educational psychology at the Max Planck Institute for Human Development in Berlin, Germany.

10.1126/science.1121139

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1. Kim, D.-H., Behlke, M.A., Rose, S.D., Chang, M.-S., Choi, S., and Rossi, J.J. (2005) Synthetic dsRNA dicer substrates enhance RNAi potency and efficacy. Nature Biotechnology, 23:222-226.

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EDITORS' CHOICE

edited by Gilbert Chin

ECOLOGY/EVOLUTION

The Dynamics of Invasions

A lupin field (left), leaf miner damage (middle), and wildflowers at Spirit Lake (right).

The 1980 eruption of Mount St. Helens in the American northwest provided ecologists with 60 square km of primary successional habitat on which to study the dynamics of recolonization. Fagan *et al.* examine the role of interactions between species in determining the course of colonization and invasion on the fresh pumice slopes of the volcano. The spatial pattern of colonization by the prairie lupin, *Lupinus lepidus*, is governed by herbivore pressure. The plants are eaten by the leaf-tying larvae (caterpillars) of several lepidopteran species, and there is evidence for thresholds in the parameter ranges of plant spatial extent and timing of initial colonization that predict whether the herbivores can halt the invasion. As well as providing fresh insight into the dynamics of successional systems, these findings are relevant to the control of invasive plants because they suggest the possibility of developing protocols for the most effective timing and spatial deployment of herbivorous control agents. — AMS

Am. Nat., in press.

CHEMISTRY

Boron Metathesis

Metal carbene (M=C) complexes have proven highly useful because of their capacity to undergo metathesis reactions, which exchange the carbene group with another doubly bonded fragment. Recently, several different researchers have prepared boron analogs (M=B) of these compounds, with a double bond between a transition metal and a monovalent boron, or borylene, center.

Kays *et al.* have shown that one such compound undergoes metathesis, much like its carbon cousin. The borylene fragment, bearing a diisopropyl amino group, is bonded to an organometallic iron center. Room-temperature exposure of this compound to a solution of triphenylphosphine sulfide ($Ph_3P=S$) quickly cleaves the P=S linkage, pairing the aminoborylene with the sulfur and leaving the phosphine coordinated to the iron. Similarly, treatment with triphenylarsine oxide ($Ph_3As=O$) produces a boron oxide compound and an arsine-coordinated metal complex. The analogous reaction with triphenylphosphine oxide ($Ph_3P=O$) is slow enough to permit isolation of an intermediate, which the authors characterized by x-ray crystallography. This intermediate shows a P-O-B linkage and lengthened Fe-B bond, suggesting that these reactions proceed by initial attack of the boron center by the electronegative oxygen or sulfur. — JSY

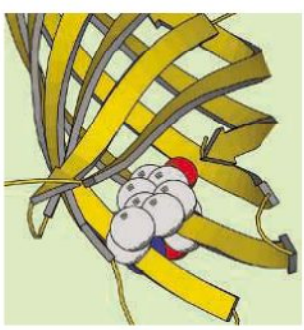
Angew. Chem. Int. Ed. 10.1002/anie.200502343 (2005).

BIOCHEMISTRY

Rescuing Glycine

The two ways to achieve structural stability of an integral membrane protein are to bundle α helices together, as in lactose permease, and to curl a β sheet (comprised of individual β strands) into a barrel, as exemplified in the family of porins. The β-barrel proteins are found in the outer membrane of bacteria and of organelles (mitochondria and chloroplasts) thought to have a bacterial heritage.

Jackups and Liang have systematically analyzed the small, but growing, data set of three-dimensional structures of β-barrel membrane proteins in order to establish the propensities of interstrand amino acid neighbors. These values have current application to improving sequence-based alignments across proteins as well as the register between β strands within proteins. In addition, motifs and antimotifs of pairs of amino acids may find application in future studies of β-barrel membrane protein biogenesis (folding, translocation, and insertion). One such motif, originally identified in soluble β-barrel proteins, is termed aromatic rescue of glycine. The curvature of the inner (and often solvent-exposed) surface of a β barrel is facilitated by glycine residues, and the energetically unfavorable exposure of the peptide backbone can be mitigated by covering the glycine with an aromatic side chain, such as is found in tyrosine and phenylalanine, from a neighboring stave of the barrel. — GJC



The side chain of tyrosine (carbon, gray; oxygen, red) is interposed between glycine and the interior of the barrel (yellow strands).

J. Mol. Biol. 10.1016/j.jmb.2005.09.094 (2005).

DEVELOPMENT

Death in the Blink of an Iris

In early development, the eye is covered with a membrane that includes blood vessels and that functions to nourish the developing lens and retina. This membrane, however, obscures the clear optical path needed for visual acuity.

Studying rats, in which the eye matures postnatally, Morizane *et al.* uncover the link between maturation of the iris and the apoptosis of blood vessels supplying the immature eye. A key moment

CONTINUED ON PAGE 749

CREDITS: (TOP) W. LL AM FAGAN; (BOTTOM) JACKUPS AND LIANG, *J. MOL. BIOL.* 10.1016/J.JMB.2005.08.594 (2005)

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is when the iris begins to move, constricting and dilating in the way that will later control the light supply to the lens and improve focus. The constricting movements place pressure on the persistent blood vessels, causing the blood supply to stop intermittently. Pharmacologic intervention that paralyzed iris movements delayed regression of the vascular membrane. The authors propose that it is the increasing intermittency of the blood flow, rather than the mechanical shear stress induced by iris movement, that signals cells of this vascular membrane to initiate apoptosis. — PJH

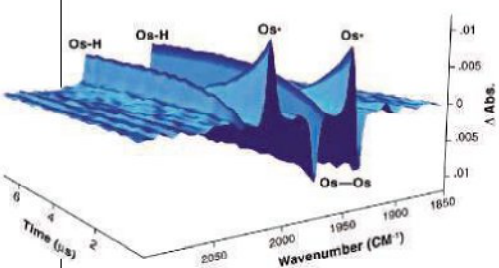
Am. J. Physiol. Regul. Integr. Comp. Physiol. 10.1152/ajpregu.00602.2005 (2005).

CHEMISTRY

Grabbing Hydrogen

The activation of normally unreactive C-H bonds by metal complexes typically proceeds through metal-to-carbon hydrogen-atom transfers. This step need not be irreversible, but direct observation of hydrogen transfers in the other direction, from carbon to metal, has been lacking.

Zhang *et al.* report time-resolved infrared spectra of this process as carried out by the osmium dimer $[Cp(CO)_2Os]_2$



Decay of the ν(CO) bands (Os•) and formation of Os-H bands.

(where Cp is cyclopentadienyl). The radical formed by homolytic cleavage of the Os-Os bond through photolysis is unlike other metal carbonyl radicals in that it does not redimerize, but instead attacks C-H bonds. The reaction of this radical with 1,4-cyclohexadiene could be followed on the microsecond time scale by the decay of the CO modes of the radical and the growth of new infrared bands assigned to Cp(CO)OsH. Electrochemical studies and thermochemical analysis revealed the driving force for this reaction: an exceptionally strong Os-H bond (82 kcal per mole) relative to M-H bonds in other metal carbonyls. — PDS

J. Am. Chem. Soc. 10.1021/ja0555724 (2005).

ASTRONOMY

Thriving in a Sea of Methane

Life on Earth relies on energy from chemical reactions or from sunlight. Chemical reactions between organic compounds and hydrogen have been proposed as a possibility for powering life forms on the surface of Titan, Saturn's largest moon.

McKay and Smith calculated the amount of energy released by reactions of hydrocarbons present in Titan's dense smoggy atmosphere with hydrogen gas. They found that there would be more than enough fuel for specialized organisms such as the methanogenic bacteria found on Earth. Although methanogens could be sustained on Titan, it might be difficult for them to consume the chemicals, because organics may not dissolve readily in the methane lakes where life may reside. — JB

Icarus **178**, 274 (2005).

VIROLOGY

Improving Child Health

Measles tends to be overlooked in the context of developing countries that have to cope with assault from many other intractable infections, yet it still accounts for more than 350,000 deaths annually in Africa, despite the availability of an excellent vaccine. Currently, 22 measles virus genotypes have been recognized, but relatively little is known about the genotypes circulating in Africa.

Muwonge *et al.*, working in Uganda, have discovered a new genotype (d10) in a 2-year study that highlights the logistical difficulties of undertaking such surveillance in a developing country. The viruses they isolated showed uniformity within the country, but significant divergence from reference strains, and were highly distinct from other known African strains, too. It is possible that measles transmission dynamics in Uganda differs from that in developed countries. Genotype surveillance in Africa should be extended not only to monitor control programs but also to describe transmission patterns and hence whether approaches to control and vaccination need revising. — CA

Emerg. Infect. Dis. **11**, 1522 (2005).

CREDITS: ZHANG *ET AL.*, *J. AM. CHEM. SOC.* 10.1021/JA0555724 (2005)

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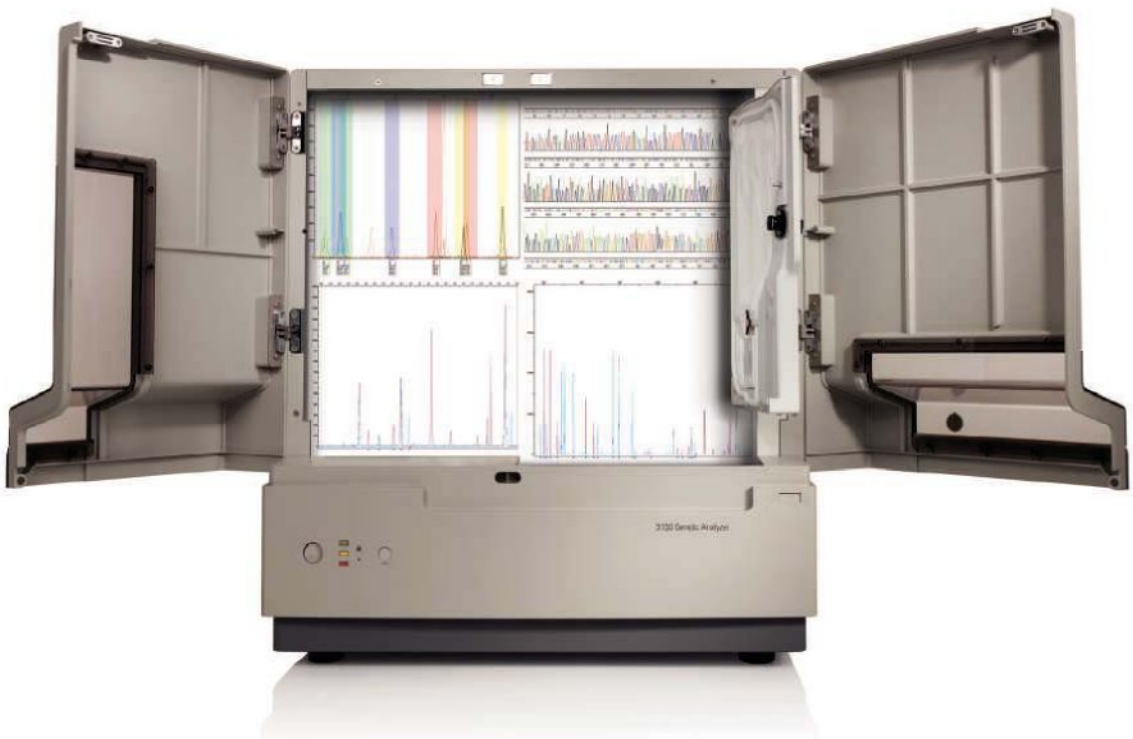

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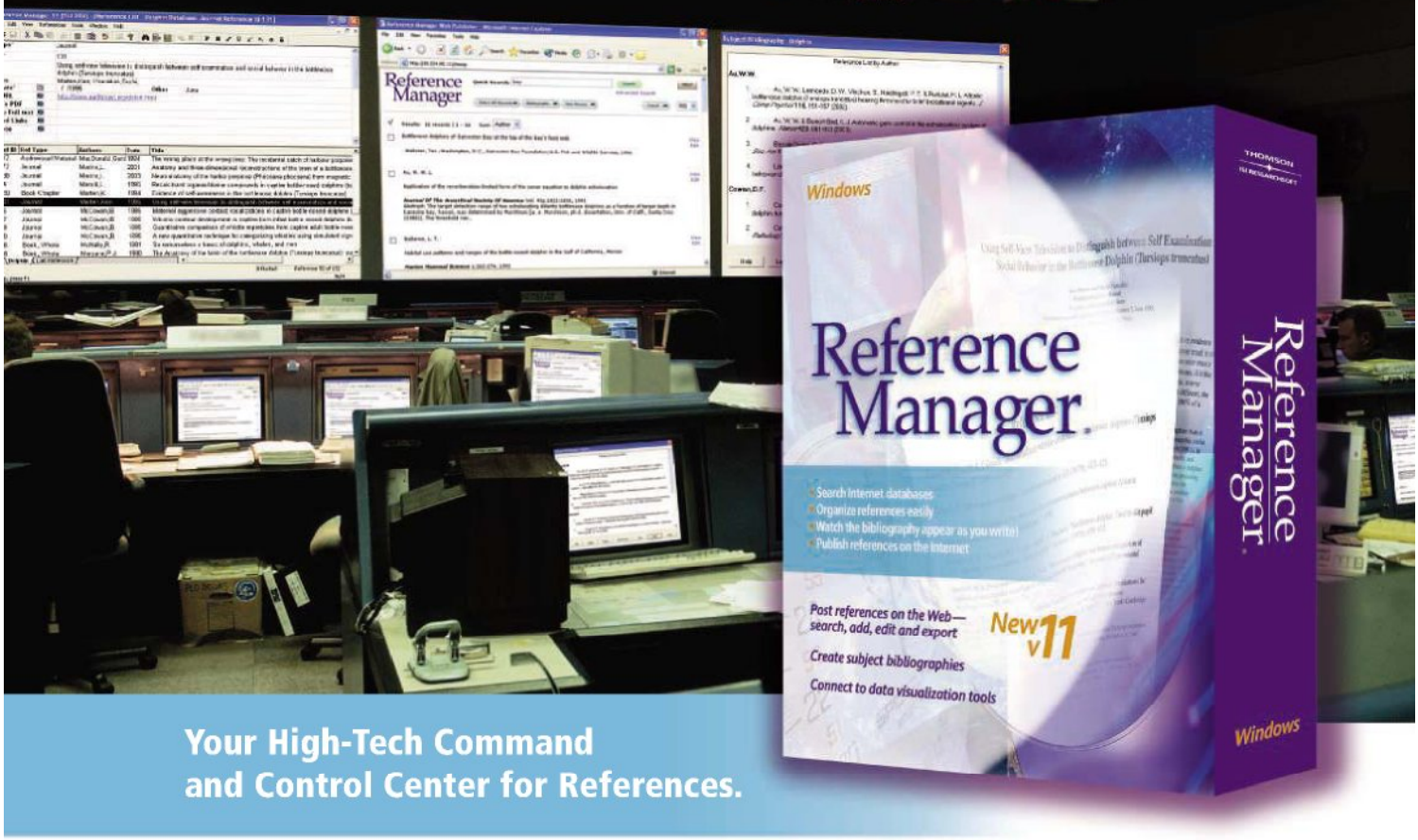

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EDUCATION

Slides on Ice

It's one thing to read that piedmont glaciers form when ice spreads out after squeezing through a narrow valley. But the concept is more likely to stick in your mind if you see the result, as in this aerial photo of sprawling glaciers on Axel Heiberg Island in northern Canada (above). Glaciers Online from Swiss teacher Jürg Alean and glaciologist Michael Hambrey of the University of Wales in the U.K. is a boon for visual learners. The pictorial introduction matches nuggets of glacier information with spectacular photos from all over the world. To distinguish cirque, outlet, and other glacier types, for example, the site spirits you from Antarctica to the Alps to the Grand Tetons. Visitors can also see how moving glaciers shape the landscape, bulldozing valleys such as Glencoe in Scotland.

www.swisseduc.ch/glaciers

RESOURCES

Supplementary Reading

Researchers who investigate dietary supplements may want to check out this list of the 25 best papers in the field from last year. The choices, selected by experts convened by the National Institutes of Health, probed questions such as the effect of high doses of vitamin C on women with diabetes. (The vitamin increased the risk of dying from heart disease.) You can download a PDF with abstracts from the papers and similar reports from the previous 5 years.

ods.od.nih.gov/Research/Annual_Bibliographies.aspx

DATABASE

Fungal Pointer

A rotting tree is a feast for corticioid, or crust, fungi. The taxonomy of these mushroom cousins can stump even experts: Over the years, researchers have minted more than 8000 names for the roughly 2000 species. Compiled by mycologists at Tartu University in Estonia and Göteborg University in Sweden, Cortbase can guide fungus fans through this baffling nomenclature, identifying which species names remain valid. The site also offers a specimen locator to pinpoint which of 147 herbaria hold representatives of particular corticioids.

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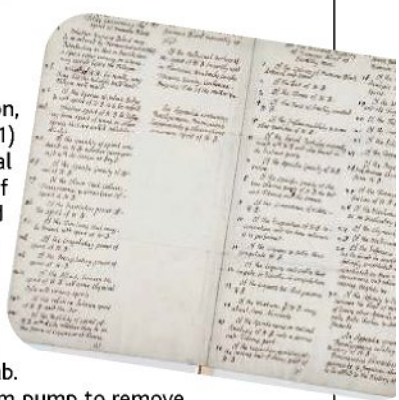
EXHIBITS

Method Man



While living through civil war and revolution, the British scientist Robert Boyle (1627–91) managed to forge the modern experimental method by investigating a broad array of topics, including human circulation and the nature of air. Learn more about Boyle's contributions and check out some of his writings at this site from the University of London.

Although he began as a nonscientific writer, Boyle proved himself a whiz in the lab. In one set of experiments, he used a vacuum pump to remove the air from a vessel containing a candle. The flame went out, and he deduced that air contained something necessary to sustain fire. At the site, you can peruse selections from 11 volumes of Boyle's papers (above, pages from his treatise on blood). A timeline puts Boyle's life and accomplishments in context with British history and intellectual developments. Boyle was one of the first scientists to publish experimental details. At a linked site, you can page through 44 years of his work diaries.

www.bbk.ac.uk/boyle

IMAGES

Just In From the Red Planet

Like any old-timer, Mars carries its share of wrinkles, scars, and blemishes, such as these marks left by collapsing lava tubes on the slopes of the volcano Ascraeus Mons (right). More eye-catching close-ups of the planet's physiognomy await at this gallery hosted by Arizona State University in Tempe. The site showcases 3 years of shots from an instrument mounted on the Mars Odyssey spacecraft that measures heat emanating from the surface. The "Live" from Mars section lets you see the images as they arrive. For more information about notable geological details, check out the weekly backgrounders. You can also browse a gallery organized by type of feature or track down any one of the 82,000 images using a clickable map of the planet.

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NEWS

This Week

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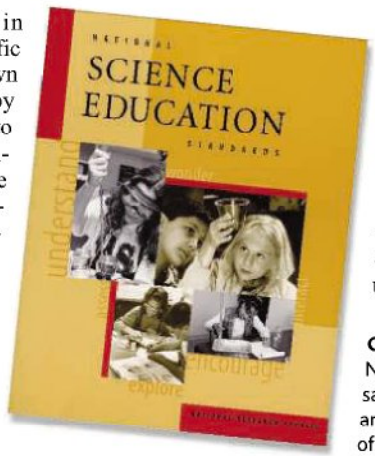
EVOLUTION

Groups Wield Copyright Power To Delay Kansas Standards

For the second time in 6 years, two U.S. scientific organizations have thrown a wrench into plans by Kansas school officials to adopt new science standards that would promote the teaching of alternatives to evolutionary theory. Observers say the move, which prevents Kansas from incorporating copyrighted materials into the state's revised standards, could indefinitely delay their adoption by forcing officials to craft substitute language.



Going by the book. The National Academy of Sciences says the Kansas draft standards are an unacceptable adaptation of its 1996 standards.

"That could take them several months," says Steven Case, a biologist at the University of Kansas in Lawrence and chair of the science standards writing committee, which has been fighting the 10-member board. "The more we can push back the implementation of the standards, the less damage they can do." Case and others see the board's proposed standards as an attempt to introduce intelligent design (ID) into school curricula.

When drafting new education standards, state officials typically borrow liberally from the National Academy of Sciences' (NAS's) *National Science Education Standards* (*NSES*) and the National Science Teachers Association's (NSTA's) *Pathways to Science Standards*. But the two organizations said in a 27 October statement that the set of standards proposed by the Kansas board "inappropriately singles out evolution as a controversial theory despite … its acceptance by an overwhelming majority of scientists," and that its definition of science "[blurs] the line between scientific and other ways of understanding." As a result, the two organizations denied Kansas the right to incorporate any of their materials into its new standards.

For example, in a section specifying what students between grades 8 and 12 need to understand about evolution, the draft reproduces the concepts listed under the same section in the *NSES*. But it also contains insertions such as " in many cases the fossil record is not consistent with gradual, unbroken sequences postulated by biological evolution." Similarly, a section on "science as inquiry" looks identical to the corresponding section in the *NSES* except for one additional statement: "[The student] understands methods used to test hypotheses about the cause of a remote past event (historical hypothesis) that cannot be confirmed by experiment." The modification seems to be aimed at "open[ing] the door for various kinds of explanations that may not be scientifically based," say NAS's Jay Labov and Barbara Schaal, who reviewed the Kansas standards.

Observers say the decision is unlikely to stop the board from adopting the standards when it meets on 8 November. But the absence of copyright permission means that the board will have to rewrite the 123-page document. Kansas education officials are working "to remove any material that would violate any copyright provisions," says Kathy Martin, one of a six-member bloc that is pushing the change. She predicts that a new version will be ready by December. "How long can we keep beating an old horse to death?" she asks.

But John Staver, a science education professor at Kansas State University in Manhattan who serves on the standards writing committee, doubts that the board will be able to complete its task so quickly. He says a similar move in 1999 by NAS, NSTA, and AAAS (publisher of *Science*) delayed implementation of new standards in Kansas by more than a year, and that the ruling majority was booted out in the meantime. "We're hoping for the same thing to happen again," says Staver. Conservatives hold four of the five board seats up for election in November 2006.

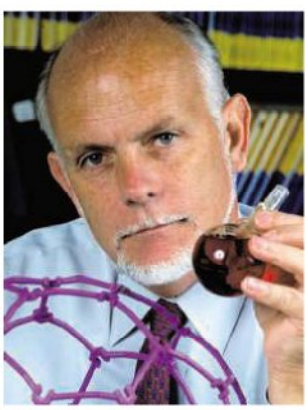
–YUDHIJIT BHATTACHARJEE

CHEMISTRY

'Grandfather of Nanotech' Dies at 62

Nanotechnology pioneer Richard Smalley, who shared the 1996 Nobel Prize in chemistry for work that launched the nanotechnology industry, died 28 October after a long battle with cancer. He was 62.

Smalley, a chemistry professor at Rice University in Houston, Texas, co-discovered carbon-60, a soccer ball–shaped form of carbon also known as buckminsterfullerene or "buckyballs." He helped persuade Congress to create the National Nanotechnology Initiative, a $1-billion-a-year federal effort that he predicted could lead to a new generation of nanotech-based drugs capable of wiping out many forms of cancer, such as the non-Hodgkin's lymphoma from which he suffered. "I may not live to see it. But, with your help, I am confident it will happen," Smalley testified in 1999.



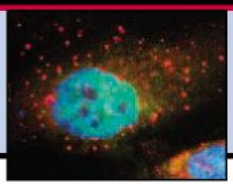
"Moses." Smalley was an early booster of nanotechnology.

"Richard was truly the grandfather of the entire field of nanotechnology," says Anna Barker, deputy director of the National Cancer Institute in Bethesda, Maryland. Jim Heath, a nanotechnology expert at the California Institute of Technology in Pasadena and a former graduate student in Smalley's lab, called him "a Moses" leading the field to the promised land.

In recent years, Smalley had also been a tireless campaigner for increased spending on new sources of energy, warning audiences that "energy is the single most important problem facing humanity today."

–ROBERT F. SERVICE

CREDITS (TOP TO BOTTOM): NATIONAL RESEARCH COUNCIL; RICE UNIVERSITY



BIODEFENSE

Critics Question Proposed Countermeasures Agency

Spurred by worries about avian influenza, a Senate panel has come up with an idea to speed the development of new drugs and vaccines against urgent public health threats such as pandemic flu and bioterror weapons. But its solution—a new research agency within the Department of Health and Human Services (HHS)—has put scientific groups on alert. They worry that its work would be secret and could duplicate existing efforts at HHS. "The creation of a new agency raises many issues," says Janet Shoemaker, public affairs director of the American Society for Microbiology in Washington, D.C.

The proposal is part of S. 1873, a bill passed on 18 October by the Senate Committee on Health, Education, Labor, and Pensions. Sponsored by Senator Richard Burr (R–NC), the legislation is in part meant to address gaps in the BioShield law passed in 2004 that provides $5.6 billion to drug companies over 10 years for procurement of biodefense drugs and vaccines. Few companies have applied for BioShield funding partly because of concerns about liability. S. 1873, which some are calling BioShield II, would protect companies from lawsuits and also offers sweeteners such as exclusive markets for countermeasures.

Top-down approach. A proposed federal agency would fund research on drugs and vaccines against natural disease outbreaks and potential bioterror weapons such as anthrax.

Perhaps the most controversial part of the legislation is its creation of the Biomedical Advanced Research and Development Agency (BARDA). Burr says the agency is modeled on the Defense Advanced Research Projects Agency, which acts quickly to fund high-risk, high-payoff research that might not pass peer review at other agencies. BARDA's focus, however, would mainly be on providing funding and coordination to reduce the time between basic research and final product.

BARDA would fund research and development on countermeasures for bioterror agents, chemical and nuclear agents, and infectious diseases that could cause natural outbreaks. In addition, it would coordinate research on biodefense and infectious disease countermeasures across the federal government—a role that no agency now fills. Although Burr's staff says BARDA's budget is still being worked out, the bill stipulates it would start out with $1 billion in 2006 from unspent BioShield 2004 funds. The legislation also calls for the National Institutes of Health to fund animal models for countermeasures research and for NIH to absorb some parts of the Armed Forces Institute of Pathology, which is to be disbanded as part of the latest defense base closings (*Science*, 2 September, p. 1472).

Scientific groups are worried about where BARDA's funding would come from at a time when research budgets are already being squeezed. The Federation of American Societies for Experimental Biology wrote Burr that it is "troubled" that the bill doesn't clarify how BARDA would differ from biodefense and infectious disease programs at NIH's National Institute of Allergy and Infectious Diseases (NIAID) and at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia. It's not clear whether the bill "will help" develop drugs and vaccines or "just adds a layer of complexity," adds Shoemaker. Observers also note that the bill does not spell out how research proposals would be reviewed or what the necessary expertise for BARDA's presidentially appointed director would be.

Another concern, say scientific and other groups, is that BARDA and a new oversight board would be exempt from the Freedom of Information Act and open-meetings laws. "Transparency is both appropriate and necessary," particularly for developing infectious-disease countermeasures, writes the Center for Arms Control and Non-Proliferation in a letter recommending that information be withheld only in cases of a threat to national security.

Burr's press secretary Doug Heye says BARDA would have "a much different role" from that of NIAID which focuses on basic research and that its reports would be public except in certain situations. Security expert Gerald Epstein of the Center for Strategic and International Studies, who testified at two hearings earlier this year on the bill, says scientists' "almost allergic reaction" to secrecy doesn't make sense in a world in which "there are folks looking at this stuff to kill us rather than help us." BARDA's overseers "can't guarantee that they'll never have to" withhold information about, say, devices to detect bioterror agents, he suggests.

An NIAID spokesperson said the institute could not comment on pending legislation. Burr, who was still revising the bill, hoped it would reach the Senate floor for a vote as early as next week. It's not clear how soon the House will take up the measure. It's also possible that the bill will be merged with measures focused on pandemic influenza, such as a provision passed last week by the Senate that would give CDC nearly $8 billion in 2006 for pandemic preparedness and President George W. Bush's new pandemic flu plan (see ScienceScope, p. 759).

—JOCELYN KAISER

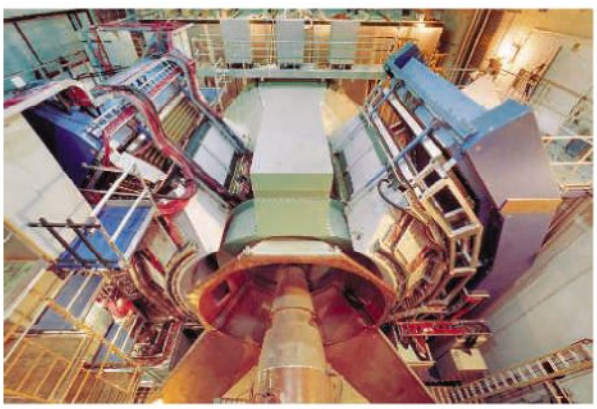
CREDIT: QUINN SOMMERS/REUTERS

NUCLEAR PHYSICS

Dueling Experiments Close In on Source of Proton's Spin

When searching for something, it can help to know where the thing *isn't*. So physicists are cheering results that eliminate one of three possible explanations of how the proton gets its spin. "This is a huge step," says particle physicist Zein-Eddine Meziani of Temple University in Philadelphia, Pennsylvania. "It's just a matter of waiting for more data until they nail it down."

For 70 years, physicists have known that the proton acts like a tiny top and possesses exactly half a fundamental smidgen of spin. But they don't know precisely where that spin comes from. The proton consists of three fundamental particles called "quarks" and a gaggle of others called "gluons." Each quark carries half a unit of spin, and theorists once thought the quarks were aligned, or "polarized," enough to give the proton most of its spin. But in the 1980s, experiments showed that the spin of the quarks accounted for only about 20% of the total.



One down. Data from PHENIX (*above*) in the U.S. and an experiment in Europe rule out one explanation of the proton's spin.

That "spin crisis" has three possible explanations. The gluons, which also have spin, might be polarized just enough to make up the missing 80%. Or it could arise from the "orbital" motion of the quarks and gluons swirling around one another. Finally, some theorists argue that both the quarks and the gluons are polarized but swirl in a way that counteracts some of the resulting rotation. Researchers measured the spin of the quarks by bouncing particles called muons off the quarks. But a gluon can split into a quark and an antiquark, and the muon can bounce off one of them. If the gluons are highly polarized, that interaction can obscure the polarization of the quarks.

Now, two teams have measured the gluon polarization. And the results, presented at a conference* in Santa Fe, New Mexico, suggest that it's small.

With help from colleagues at the Japanese research agency RIKEN, researchers at Brookhaven National Laboratory (BNL) in Upton, New York, collided beams of protons polarized to spin along like little American footballs thrown using either the right or left hand. Experimenters studied the resulting spray of particles using the PHENIX particle detector. As they switched the relative polarizations, they tracked changes in the number of particles called pions to deduce the gluon polarization, says BNL's Gerry Bunce. The results rule out a large gluon polarization that masks the quark polarization, he says, but don't yet reveal whether the gluons provide the missing spin.

Meanwhile, as in earlier experiments, researchers with the COMPASS experiment at the European particle physics laboratory CERN near Geneva, Switzerland, smashed polarized muons into a target containing polarized protons. This time they used judicious data "cuts" to identify collisions in which a gluon splits into a quark and an antiquark—the same ones that may have muddled measurements of the quark polarization. That trick enabled the physicists to measure the polarization of the gluons. It accounts for no more than 10% of the proton spin, says particle physicist Jan Nassalski of the Soltan Institute for Nuclear Studies in Warsaw, Poland.

Researchers debate which result is more definitive. The PHENIX experimenters use the unknown structure of one proton to probe the structure of the other, says CERN experimenter Gerhard Mallot, "and I think that's a disadvantage because you have unknown squared." But Werner Vogelsang, a theorist at BNL, says the COMPASS measurements rely on shakier theoretical assumptions and apply only to gluons moving with a particular momentum within the proton.

All agree that the two experiments are complementary and that the uncertainties will shrink with more data. Within a few years, most expect to know at last what's whirling within the proton. —ADRIAN CHO

*Particles and Nuclei International Conference, 24–28 October 2005, Santa Fe, New Mexico.

CREDIT (LEFT): BROOKHAVEN NATIONAL LABORATORY

ScienceScope

High Court to Rule on Patent Limits

Can researchers patent a scientific fact? This week, the U.S. Supreme Court agreed to review the question in a dispute between diagnostics makers. A 1986 patent belonging to University of Colorado–affiliated Metabolite Laboratories in Colorado covers a technique to measure homocysteine, a marker for vitamins in the blood. The claim at issue is the correlation scientists discovered—and patented—between homocysteine concentrations and vitamin levels.

In 1999, Metabolite Laboratories, which owned the patents, sued LabCorp, which had developed a rival test that relies on that correlation, for infringement. LabCorp says that Metabolite's patent, if legitimate, means companies can "claim monopolies over basic scientific facts." Metabolite says its discovery is its rightful intellectual property and not a law of nature, which cannot be patented. "[Y]ou'd call it a guideline of nature more than a law of nature," says Metabolite attorney Glenn Beaton of Gibson, Dunn & Crutcher in Denver, Colorado.

—ELI KINTISCH

IP Poses Quandary for Institute

The California Institute for Regenerative Medicine (CIRM) was launched last fall amid promises that the $3 billion stem cell research enterprise would generate up to $1 billion in royalties and other revenue for the state. But officials are still resolving questions about how to divvy up such intellectual property (IP) claims.

This week, state Senator Deborah Ortiz (right) asked experts to describe various possible arrangements. One model discussed is that of the International AIDS Vaccine Initiative, in which companies agree to lower treatment costs in exchange for IP rights to any new medicines.

The institute's financing is also in limbo. CIRM officials are trying to raise $55 million in bridge funding pending the resolution of lawsuits that have stalled a bond issue. But tax-free bonds, the preferred route, could jeopardize the state's ability to collect royalties.

Ed Penhoet of the CIRM advisory board says the institute hopes to have an IP policy in place by February. —CONSTANCE HOLDEN

SCIENTIFIC MISCONDUCT

MIT Terminates Researcher Over Data Fabrication

A rising star at the Massachusetts Institute of Technology (MIT) in the hot field of RNA interference (RNAi) was dismissed last week after admitting that he had fabricated and falsified data in grant applications, submitted manuscripts, and one published paper, the university reported in a statement. The California Institute of Technology (Caltech) in Pasadena has now begun reviewing two papers published by the researcher, Luk van Parijs, 35, when he was a postdoc there. Harvard Medical School and Brigham and Women's Hospital, where Van Parijs was a graduate student, is also scrutinizing his early work.

"I thought Luk was an excellent scientist and truly cannot understand why he would fake anything," wrote Caltech president David Baltimore in an e-mail message to *Science*. Van Parijs was a postdoc in Baltimore's lab in the late 1990s. Van Parijs did not reply to an e-mail message seeking comment.

Graduate students and postdocs in Van Parijs's lab first approached MIT administrators in August 2004 with allegations of research misconduct, says Alice Gast, MIT's associate provost and vice president for research. "There were data that they could not verify the origins of," says Gast. The university launched an investigation, put Van Parijs on paid leave, pulled his lab Web site off the MIT server, and reassigned his lab members to other faculty. A copy of Van Parijs's home page from 2003 shows that his lab had 17 members.

Gast oversaw the investigation, which was conducted by investigators whose names have not been made public. She declines to say which of 22 papers Van Parijs co-authored during his 5 years at MIT contains allegedly falsified information, nor would she quantify the number of grants or manuscripts at issue. MIT, she says, is working with the co-authors to retract the suspect published paper.

Van Parijs, a prominent and prolific young researcher in RNAi, was trying to use the method, which can alter gene expression, as a tool for studying normal physiology and disease. The applied nature of his work may have made it more difficult to detect problems, because it was less likely to match other research exactly, says Thomas Tuschl, a basic RNA biologist at Rockefeller University in New York City. "If somebody picks a gene and turns it off, it's only the people who already have a knockout who can say [if] that's the wrong thing," he says.

MIT's findings have put many of the top journals in which Van Parijs published on alert. *Immunity*, which ran seven articles by him, "will be looking into these cases in detail," said Lynne Herndon, the president and CEO of *Immunity*'s publisher Cell Press, in a statement. Staffers at both *Immunity* and the *Journal of Immunology* say they learned of the misconduct case from reporters.

MIT hasn't yet returned any of Van Parijs's grant money to the National Institutes of Health (NIH). But the university is now beginning to weigh that possibility. "That's definitely one of the next steps," says MIT spokesperson Denise Brehm.

Since fiscal year 2001, Van Parijs had won NIH grants totaling at least $1.2 million. But two of his three grants expired in August 2004, and the third would have expired in August 2006. —JENNIFER COUZIN

SCIENCE AND THE LAW

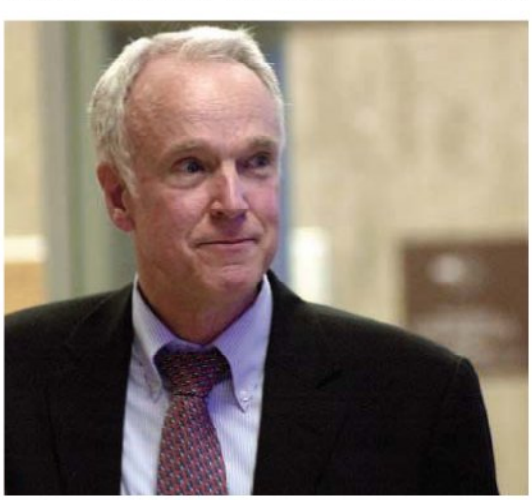
Thomas Butler Loses Appeal, Vows to Fight On

Texas physician and microbiologist Thomas Butler suffered another defeat last week in a legal battle that has already cost him his freedom, his career, and more than $1 million in legal fees. Last week, a three-judge panel on the U.S. Court of Appeals for the Fifth Circuit in New Orleans—operating temporarily from Houston—unanimously upheld Butler's conviction and 2-year prison sentence for illegally shipping bacteria to Tanzania and defrauding his former employer, Texas Tech University Health Sciences Center in Lubbock.

Although "very disappointed," Butler is "determined to continue his appeal" and restore his honor, says his lead attorney, George Washington University law professor Jonathan Turley. Meanwhile, supporters are trying to help the 64-year-old researcher find a job once he is released from federal prison on 2 January.

Butler's troubles began in January 2003, when he reported that 30 vials of plague bacteria were missing from his lab. His statements triggered a massive FBI operation and a nationally televised bioterror scare in Lubbock, a college town in western Texas. Butler was eventually charged with lying to investigators, mishandling plague samples, defrauding Texas Tech, and tax evasion. Although a jury acquitted him on most of the plague-related charges, he was convicted of 47 offenses and received a 2-year sentence (*Science*, 19 March 2004, p. 1743).

Butler's lawyers argued that lumping the charges related to plague with allegations on financial wrongdoing may have prejudiced the jury, that Butler should have had the right to subpoena internal e-mails and take depositions from four witnesses in Tanzania, and that prosecutors offered no evidence that Butler willfully violated export rules when he sent plague cultures to Tanzania via FedEx.



Back to work? Thomas Butler hopes to find a job after completing his sentence.

CREDIT: J. BROCK/AVALANCHE-JOURNAL; JIM WATKINS/AP PHOTO

Turley says he's "frankly astonished" by the ruling from what is generally considered one of the most conservative appeals courts in the country. But he expects Butler, now in prison in the Federal Medical Center in Fort Worth, Texas, to continue the fight, to the Supreme Court if necessary.

Last week, members of the National Academy of Sciences's Committee on Human Rights, chaired by Duke University's Peter Agre, a Nobel laureate and ardent supporter of Butler, discussed ways to help him rebuild his ruined career. But as a convicted felon who gave up his medical license, Butler faces an uphill battle, Agre says.

Stanford microbiologist Stanley Falkow, another prominent Butler defender, says his efforts to find Butler a job have failed to bear fruit. "Short of him leaving the country, it's going to be very difficult," Falkow says. Butler "really wants to work again," says his wife, Elizabeth Butler. "I think work will help him heal." —MARTIN ENSERINK

NEUROSCIENCE

Genes That Guide Brain Development Linked to Dyslexia

Genetic variations that cause miscues in brain development may play an important role in reading disabilities such as dyslexia, according to research presented last week at a meeting of the American Society of Human Genetics in Salt Lake City, Utah.

"Before these studies, no one has really known what's going on" in the brain to cause dyslexia, says Juha Kere, a molecular geneticist at the Karolinska Institute in Stockholm, Sweden, and leader of one of the studies. Taken together, Kere says, the new work strongly suggests that dyslexia results from faulty neural connections formed early in life.

People with dyslexia have reading impairments despite normal intelligence. The problem affects up to 17% of the population and tends to run in families, pointing to a strong genetic component. Geneticists have recently implicated several genes, but little has been known about how they might contribute to the disorder.

In one new study, a collaboration of 20 researchers led by Haiying Meng and Jeffrey Gruen of Yale University School of Medicine homed in on a region of chromosome 6 that had been fingered previously. Using DNA from 536 people with a dyslexic in their families, the researchers tracked 147 single-nucleotide polymorphisms (SNPs), spots where the genetic code differs by one letter among individuals. Searching for SNPs that tend to have one "spelling" in people with reading impairments and another spelling in normal readers, the researchers found a disproportionate number of such SNPs in a gene called *DCDC2*. They also found that about 17% of dyslexics were missing a short stretch of DNA within *DCDC2*. Everyone who had this deletion had dyslexia, Gruen says.

Analyses of cadaver brains revealed high levels of *DCDC2* expression in brain regions used during reading. And when the researchers used a technique called RNA interference to dampen *DCDC2* activity in fetal rats, newly born neurons didn't migrate to their proper positions in the cerebral cortex, the team reported at the meeting and online this week in the *Proceedings of the National Academy of Sciences*. This suggests that certain variations of the *DCDC2* gene may damage development of the neural circuits normally used for reading, says Gruen. People who inherit those variations probably compensate by using less efficient circuits for reading, he says.

Also at the meeting and in a paper published on 28 October in *PLoS Genetics*, Kere and colleagues reported evidence linking a gene on chromosome 3 called *ROBO1* to dyslexia. In one man with dyslexia, the team found that the *ROBO1* gene had been disrupted by a freak genetic accident: a piece of chromosome 8 wedging itself into chromosome 3. Kere's team also found reduced *ROBO1* activity in 21 dyslexic individuals from a large Finnish family. The fruit fly version of *ROBO1* helps shape neural connections between the two sides of the brain during development, and Kere says such connections may be impaired in people with dyslexia.

Reading right? Genetic variations that alter neural wiring may contribute to dyslexia.

A third candidate dyslexia gene called *KIAA0319*, first described by Julie Williams of Cardiff University, U.K., and colleagues in February in the *American Journal of Human Genetics*, may also play a role in brain development, according to work presented by Anthony Monaco at the Wellcome Trust Centre for Human Genetics in Oxford, U.K., and colleagues.

Gruen predicts that the new work will quickly lead to genetic tests for dyslexia susceptibility. "If we can identify kids early, we can get them into [classes] tailored to their problem," he says. But others aren't so sure. Monaco and Williams, for example, say they've failed to find an association between *DCDC2* and dyslexia in their British populations. Kere, on the other hand, has a paper in press at the *American Journal of Human Genetics* replicating the *DCDC2* link in a German population. Everyone agrees that more work is needed to resolve the discrepancies. One possibility, Gruen says, is that different genes are more important for dyslexia susceptibility in different populations. –GREG MILLER

CREDIT: SCOTT MOUR HEWITT/GETTY IMAGES

ScienceScope

Third Flood for Grand Canyon

The U.S. Geological Survey and its partners that care for the Grand Canyon are planning to flood the canyon for the third time in 10 years to preserve sandbars along the Colorado River and study the river. Over the years, the Glen Canyon Dam has trapped sediment and altered water flow, changing the river environment. As a follow-up to previous dam releases, hydrologists will lower the flow later this year and then increase it in early spring to push sand into sandbars throughout the canyon. Previous releases have not spread sand as uniformly as officials wanted.

–ELIZABETH PENNISI

Azerbaijani Physicist Held

Human-rights activists are lobbying for the release of a prominent Azerbaijani physicist detained last week by authorities in an ongoing wave of arrests. Eldar Salayev, 72, former head of the country's Academy of Sciences, is among roughly three dozen people who have been arrrested for plotting to overthrow the government.

Salayev's son, Elman, is a leader of the Azerbaijan Democratic Party, which opposes the authoritarian government of President Ilham Aliyev. The elder Salayev has been an outspoken critic of the government, but his lawyer says he sought political change through democratic means.

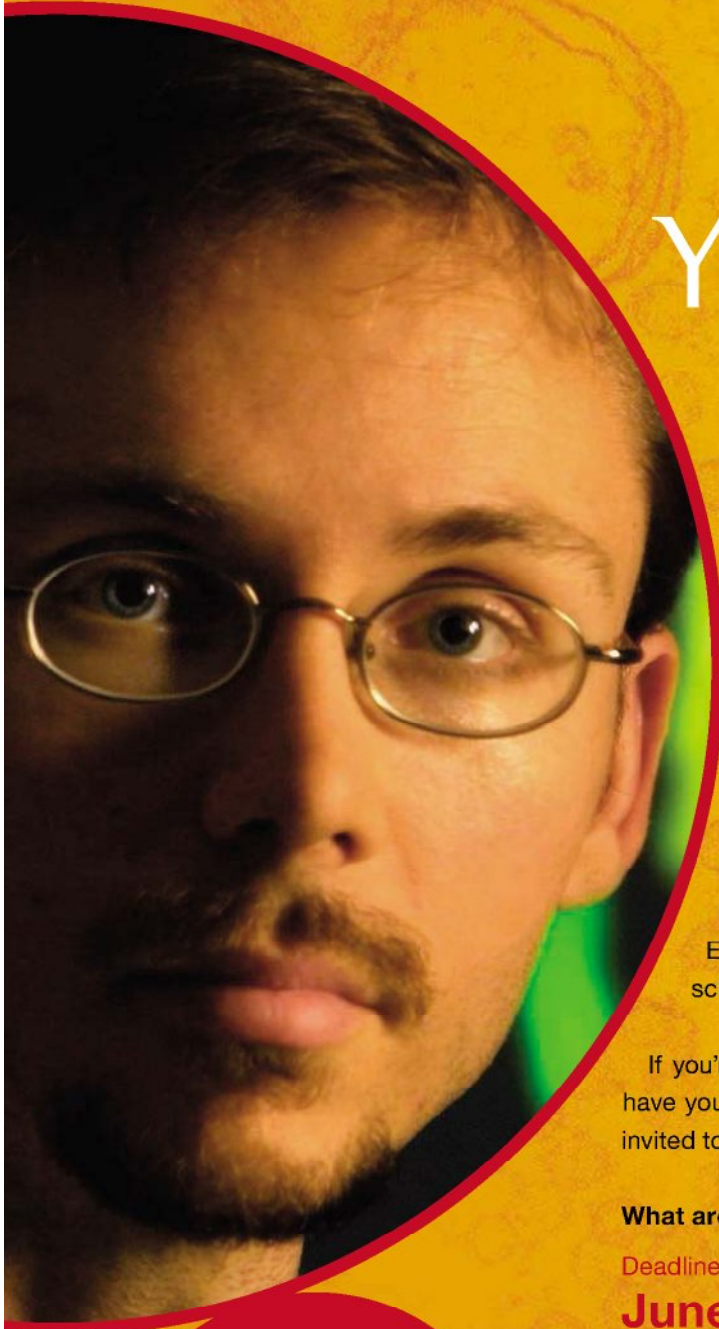
–BRYON MACWILLIAMS

Bush Unveils Pandemic Flu Plan

U.S. President George W. Bush this week announced that he will ask Congress for $7.1 billion in emergency funds to help prepare the nation for an influenza pandemic. Speaking at the National Institutes of Health, Bush noted growing concerns that the H5N1 avian influenza virus could acquire the ability to be transmitted from human to human. "If we wait for a pandemic to appear, it will be too late to prepare," he said.

Bush is asking for $2.5 billion to stockpile antiviral drugs and vaccines. In addition to funding for other countries and local preparations, Bush wants to spend $2.8 billion on cell-based vaccine technology to prepare doses against a pandemic strain in a hurry if needed. The speech comes a week after the Senate approved $8 billion for pandemic flu preparedness, and Congress is expected to meld its wishes with the president's request.

–JOCELYN KAISER



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PATENTS

Antibody Drug Dispute Ends in $255 Million Cash Payout

Developers of a new drug for arthritis last week ended a 2-year dispute over royalties, leading to one of the biggest-ever lump-sum payments on record to academic scientists. The payoff came after a U.K. biotech firm, Cambridge Antibody Technology (CAT), withdrew a suit against its giant partner Abbott in Abbott Park, Illinois.

The settlement between CAT and Abbott will release a total of $255 million in royalties, to be split between the U.S. nonprofit Scripps Research Institute, the U.S. biotech firm Stratagene, and the U.K. Medical Research Council (MRC), which helped fund early studies. The deal cuts future royalties Abbott must pay on Humira, a blockbuster anti-inflammatory drug made with a process patented by Scripps and MRC scientists.

"I expect it will be one of the single largest academic licensing transactions in the United Kingdom's history and would be very high on the list of North American licensing transactions," says Ashley Stevens, technology transfer director at Boston University. Emory University in Atlanta, Georgia, holds the record, though: In July, it sold the rights to AIDS drug Emtriva for a one-time payment of $525 million. U.S. universities in 2003 garnered $1.3 billion in licensing revenue from science discoveries, according to the Association of University Technology Managers.

At the heart of the CAT-Abbott deal is a technique to create human antibodies to order. Previously, animal cells were used to derive antibodies for medical therapies, and as a result, candidate drugs triggered an immune attack by the patients' own cells. Competing labs at MRC and Scripps published landmark papers on techniques to derive human monoclonal antibodies in 1989 (*Science*, 8 December 1989, p. 1275; *Nature*, 12 October 1989, p. 544). Nine years later, after shelving a patent dispute, the teams joined forces under the auspices of CAT. Abbott and CAT then partnered to produce Humira but eventually found themselves at odds in 2003 over profits; Abbott attempted to reduce the royalty rate to the inventors, citing contract provisions, and CAT sued. The parties settled out of court last week.

Doctors have prescribed Humira to more than 110,000 patients worldwide for rheumatoid arthritis. In addition, drugs based on the MRC-Scripps antibody technique are in clinical trials for Crohn's disease and ankylosing spondylitis.

"You like to see academic research ultimately go to products to help people," says chemist Richard Lerner, a co-inventor and now president of Scripps. "Humira is a very good product," adds co-inventor Gregory Winter, an MRC researcher at the University of Cambridge, U.K., in part because it is formulated in a way that permits patients to administer injections themselves. "I think [analysts] believe the market will go to about $1.6 billion."

MRC and Scripps are poised to receive $191 million and $34 million respectively, with roughly $45 million more in the offing. Lerner is entitled to a quarter of Scripps's take; the U.K. inventors to 10%–15% of MRC's share. None has decided how the money will be used. San Diego, California–based Stratagene will receive $24 million for related patents. —ELI KINTISCH

ITALIAN UNIVERSITIES

Government Wins Fight to Modernize Academic Appointments

ROME—After almost 2 years of debate, Italy's Parliament approved a law last week to reform the status and recruitment of academic staff and bring the university system in line with those of other leading nations. The most dramatic change will be the elimination of the *ricercatore* position, a tenure-track job for young researchers, currently numbering 20,000. The law will also switch professorial appointments from a local to a national system and allow universities the autonomy to take on contract research projects and make ad hoc academic appointments.

The government took action this autumn after the bill risked running aground under the weight of hundreds of amendments. On 28 September, University Minister Letizia Moratti called on the Senate to give the bill a vote of confidence. Designed to free up the bill's progress, the appeal passed the next day, with government supporters blaming the opposition for obstruction tactics. But opponents complained of a "coup," and the college of university rectors (CRUI), an unyielding critic, declared the action an "unacceptable forcing of parliamentary practice." When the bill returned to the Camera—Parliament's lower house—on 25 October, opposition delegates walked out in protest. The bill was passed.

The position of *ricercatore*, which the bill will phase out by 2013, was introduced in 1980 to boost university research. In reality, many *ricercatori* were overloaded with teaching duties while others remained in the role for an academic lifetime. Under the new law, young researchers will be employed on 3-year contracts and can complete only two contracts before they must apply for an associate professor position.

CREDIT: CONFERENZA DEI RETTORI DELLE UNIVERSITÀ ITALIANE

Future imperfect. Rectors' leader Piero Tosi wants more reform.

The new law will also reform the *concorsi* system, in which universities set up panels to vet candidates for promotion to associate or full professor. The *concorsi* have often been attacked for favoring in-house candidates. Moratti plans to combat this "localism" by returning to national appointment competitions abandoned in reforms 7 years ago. Successful candidates will be put on a list from which universities can choose individuals to apply to fill their posts.

Another aspect of the law covers new rights for universities to draw up contracts with businesses and other bodies to fund research. And to combat brain drain, says Moratti, they will be able to directly appoint candidates from abroad—Italian nationals or otherwise—to associate and full professorships. Researchers from industry may also be named temporary professors.

Although Moratti is confident that the provisions will benefit young researchers and "bring the Italian system up to that of the most advanced countries," there remain many opponents. During the bill's progress, CRUI, for one, called for even greater university autonomy, researcher assessment to ensure a meritocratic system, better career paths for young researchers, and guarantees of adequate funding. According to CRUI President Piero Tosi, approval of the new law is unfortunate because basic questions about the future of the universities are left "unresolved." —SUSAN BIGGIN

Susan Biggin is a writer in Trieste, Italy.

Once dismissed, myths are winning new attention from geologists who find that they may encode valuable data about earthquakes, volcanoes, tsunamis, and other stirrings of the earth

Tracking Myth to Geological Reality

Big wave. Battles between mythical beings, such as a thunderbird snatching a whale in its talons, may describe ancient tsunami in the Pacific Northwest.

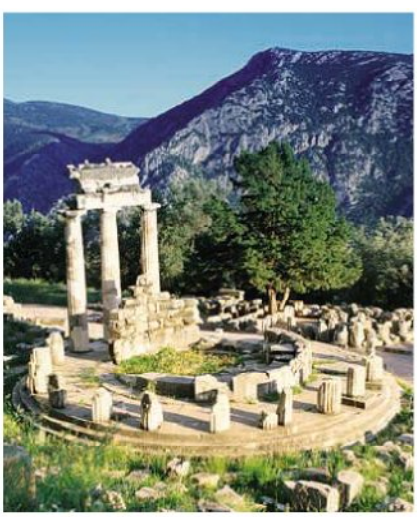
SEATTLE, WASHINGTON—James Rasmussen, owner of a funky used-record store called Bud's Jazz, and Ruth Ludwin, a seismologist at the University of Washington, Seattle, make an unlikely professional team. Late last year, they were walking down the beach near the bustling Fauntleroy ferry dock, searching for a reddish sandstone boulder. Native American legends—Rasmussen belongs to the local Duwamish people—say the boulder is haunted by *a'yahos*, a spirit with the body of a serpent and the antlers and forelegs of a deer. Old folks used to say not to look in the direction of *a'yahos* because it could shake the ground or turn you to stone. "It was not at all clear to me what my granddad was talking about when he said you should be careful as you travel through here along the shore," said Rasmussen. "Then I heard the scientific evidence, and it got me thinking about the old stories."

The evidence is this: In the 1990s, geophysical images and excavations revealed a huge, hidden fault traversing Seattle. Disturbed soils and other evidence show that 1100 years ago, it produced a quake that would level Seattle today. Scientists agree that the fault could slip again at any time, toppling buildings and elevated highways. The city's infrastructure is now being reinforced for disaster. Ludwin, Rasmussen, and others have documented at least five Seattle-area legend sites related to shaking, including the boulder, all aligned along the fault near old landslides and other signs of seismic violence. They conclude that the threat was encoded in folklore long before scientists uncovered physical signs.

More and more geoscientists are willing to combine their work with such stories these days, in a budding discipline called geomythology. Volcanologist Floyd McCoy of the University of Hawaii, Manoa, says discussing myth has traditionally been "a good way to sink your own credibility"; it can put you on the list with flaky Atlantologists and other amateur zealots. But, says McCoy, "I'd be a fool to write it all off. There is a new realization that some myths have something to say." Myths can sometimes alert researchers to previously unheeded geohazards; in other cases, where science has demonstrated the danger, legends "enrich the record" and reinforce the fact that people lie in harm's way, says paleoseismologist Brian Atwater of the U.S. Geological Survey (USGS) in Seattle, who has spearheaded many studies of seismic events in the Pacific Northwest. The trick is teasing out which myths carry kernels of truth that can be connected to hard data.

Deities of flood and fire

The movement traces in part to the 1980s, when scientists realized that the slow march of geologic time is sometimes punctuated by biblical-scale catastrophes, such as the giant meteorite that wiped out dinosaurs 65 million years ago. After this was accepted, some (usually those with tenure) felt freer to wonder if near-universal myths of great floods and fires implied that such disasters also have punctuated human time. In the 1990s, Columbia University marine geologists Walter Pitman and William Ryan argued that rising Mediterranean sea levels following the last deglaciation topped what is now the Bosporus Strait and roared into the Black Sea 7600 years ago, serving as the original inspiration for the biblical flood. Their work triggered sharp criticism and a torrent of research, resulting in growing acceptance of some sort of Black Sea flooding (*Science*, 22 September 2000, p. 2021). Whether the book of Genesis somehow grew from this is a further step, admits Ryan, who presented his latest findings at the International Geoscience Program in Istanbul, Turkey, in early October.



Apollo's voice. Intoxicating gases seeped through a fault below the oracle at Delphi.

Recent studies on more local disasters have raised the field's stock, with geoscientists connecting myths to past disasters in North America, the Mideast, Africa, Europe, and the Pacific. For example, Ludwin's study on the Seattle fault came out this year in *Seismological Research Letters*, along with a paper in which she discusses dozens of aboriginal stories about times when the ocean along British Columbia, Washington, and Oregon rolled up in great waves, carrying away coastal villages. Native people often described these events as a battle between a great whale and a thunderbird.

Paleoseismologists have a modern explanation: Quaking along the offshore subduction zone has produced at least a dozen huge tsunamis at intervals of 200 to 1000 years, as shown by shore deposits including inland sand sheets and mud that buried native camps. The most recent wave is dated through tree rings and other evi-

CREDITS (TOP TO BOTTOM): NORDICGETTY IMAGES; AMERICAN MUSEUM OF NATURAL HISTORY (INSET); PETER ADAMS/GETTY IMAGES

dence to January 1700; scientists agree the next can come any time.

The utility of myth became clear in the Indian Ocean tsunami of 2004. While up to 300,000 people are thought to have died, the indigenous seafaring Moken people of Thailand almost all survived. Their traditions warn that when the tide recedes far and fast—as happens before tsunamis—a man-eating wave is coming, and everyone should run. They did.

Patrick Nunn, a geoscientist at the University of the South Pacific in Suva, Fiji, believes such stories can be harnessed to find other hidden geohazards. He currently has a grant from the French government to collect tales that might pinpoint islands where scientists should look for warnings of earthquakes, volcanoes, or catastrophic landslides not included in written records. These include common motifs in which deities "fish up" islands from the water and sometimes throw them back. Nunn thinks such tales may encode sudden uplifts, subsidences, or flank collapses of islands, and he has already confirmed that sinking islands are not just myths. He has correlated at least a half-dozen stories with actual land masses seen by early European seafarers but which are now gone; a few were never charted but have since been located just under the waves, exactly where the stories said they were.

Nunn's studies have also turned up a surprise. People on the volcanic island of Kadavu, Fiji, have a suggestive legend about a big mountain that popped up one night, and locals say they have heard rumbling from the main cone recently. In 1998, Nunn and others investigated the volcano but decided on preliminary evidence that it had not erupted for 50,000 years. The island has been inhabited for only 3000 years, so they concluded that the myth was imported. Months later, a new road cut revealed pot shards under a meter-deep layer of ash. "The myth was right, and we were wrong," says Nunn.

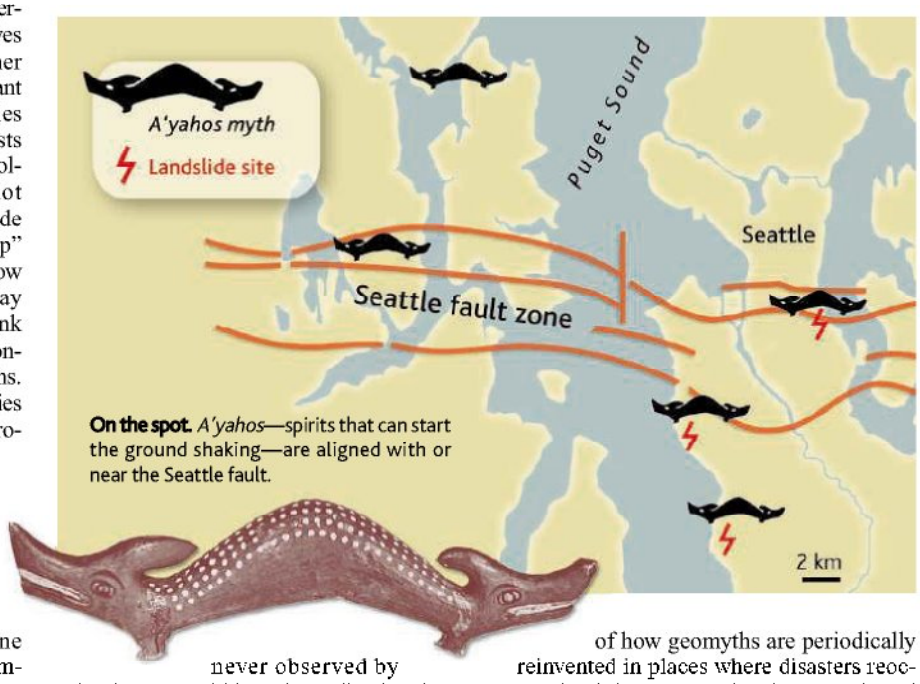
Myths may provide unusually precise tools in the Pacific because some are tied to royal genealogies that can be roughly dated. In Hawaii, where the genealogies go back 95 generations, archaeologist Bruce Masse of Los Alamos National Laboratory in New Mexico has compiled stories of battles between the fire deity Pele and others that seem to relate to volcanic eruptions; the reigns of kings at the time of the "battles" correlate within a few decades to radiocarbon dates of burned vegetation under lava sheets. Other tales apparently record celestial events. One, said to have taken place during the reign of King Kakuhihewa, narrates a human sacrifice at dawn interrupted by giant owls who fly across the sun. When Masse lined up the number of generations with recent NASA tables that calculate times of past events, he hit a match: A rare solar eclipse took place over Hawaii precisely at sunrise on 10 April 1679.

Myth has also figured in work at Nyos, a crater lake in Cameroon that exploded and killed 1700 people in 1986. The disaster was at first a mystery, with no signs of volcanic eruption. Scientists finally figured out that carbon dioxide bubbling from deep rocks had slowly built up in the water, then burst out and suffocated all living things nearby—a phenomenon never observed by scientists. It could have been dismissed as a one-time fluke except for the fact that the region is full of stories about haunted lakes that rise, sink, or blow up. Anthropologist Eugenia Shanklin of The College of New Jersey in Trenton, who collected the stories, says many local people have taboos against living near lakes and instead dwell on high ground. Scientists now know that gas buildup affects at least one other lake in the region, Lake Monoun, as well as giant Lake Kivu in east Africa, which has 2 million people living on its shores. The myths "helped tell us it happened before, and it will happen again," says geochemist William Evans of USGS in Menlo Park, California, who is working to remove gas now rebuilding in Nyos and Monoun.

Next year, the Geological Society of London will publish *Geology and Myth*, a collection of papers by Shanklin, Nunn, and others. Co-editor Luigi Piccardi, a structural geologist at the National Research Council of Italy, says he hopes it will lead colleagues to take the field more seriously.

Among other work, Piccardi has studied a cataclysmic 493 C.E. appearance at southern Italy's Monte Sant'Angelo by the Archangel Michael, said to have left his footprint in the rocks—code, Piccardi says, for a big, previously unauthenticated earthquake. In the late 1990s, Piccardi found ample physical evidence for the event, including a dramatic fault scarp in the floor of the popular shrine to the apparition, long hidden until it was uncovered in archaeological excavations—the apparent "footprint." In 2001, the National Institute of Geophysics and Volcanology in Rome upgraded the area to seismic high risk. This may also be an example of how geomyths are periodically reinvented in places where disasters reoccur: The shrine was previously an oracle and supposed entry to the underworld dedicated to the Greek seer Kalchas, who is mentioned in *The Iliad*. Piccardi's description of the shrine is in press at *Tectonophysics*. Piccardi is currently studying the possibility that many ancient sites of worship and miracles are over active faults, on the theory that past rumblings and cracking have been transmuted into visits by monsters and gods.

One such example is the oracle at Delphi, Greece. Here, priestesses were said to enter prophetic trances by inhaling the breath of the god Apollo from a magical chasm; people came from around the ancient world to hear their words. While the oracle was indisputably real, classical scholars wrote off the chasm as an invention—until geologist Jelle de Boer of Wesleyan University in Middletown, Connecticut, and archaeologist John Hale of the University of Louisville in Kentucky published a series of papers on the oracle over the past few years. De Boer and Hale showed that the ruins of Delphi lie over the juncture of two faults that conduct up psychoactive hydrocarbon gases through a spring, exactly as described in ancient accounts. (Why some prophecies were uncannily accurate is another question.) This



On the spot. *A'yahos*—spirits that can start the ground shaking—are aligned with or near the Seattle fault.

CREDIT: NATIONAL MUSEUM OF THE AMERICAN INDIAN/IMAGE ADAPTED FROM R. LUDWIN (MAP)

summer, de Boer and Hale visited the partially excavated ruins of the oracles of Apollo at Klaros and Didyma in southwest Turkey and detected hydrocarbon gases there too.

From story to data

The process of translating myth into geology, or vice versa, can be murky, but Elizabeth Barber, a professor of linguistics and archaeology at Occidental College in Los Angeles, California, believes it can be done scientifically. In the recent book *When They Severed Earth From Sky: How the Human Mind Shapes Myth*, she argues that transmutations of reality into myth take predictable courses, with natural forces often turned into supernatural beings (*Science*, 27 May, p. 1261). Some examples seem straightforward. A story from the Klamath people of Oregon about a battle between the chiefs of Above World and Below World is faithful in every geologic detail to the volcanic explosion of Mount Mazama and the formation of Crater Lake in its place, from the rain of burning ash and rock to many years of rainfall afterward that eventually filled in the crater—a process that started 7000 years ago. Other legends are more confusing. These include a hypothesis that the pillars of cloud and fire that guided the Hebrews from Egypt came from the 1625 B.C.E. volcanic eruption of Thera in the Mediterranean. Here, mismatches between dates of the events and problems with the Hebrews' route lead Barber to think the account is conflated from several real but distinct events. "The question is how often and in what cases you can take it back literally," she said.

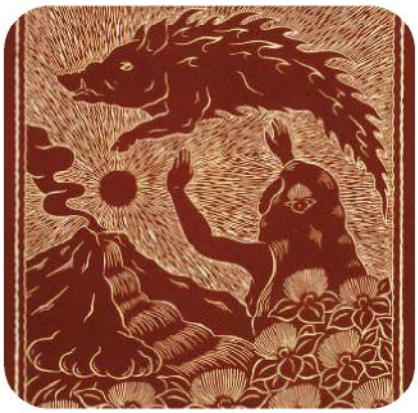
Other researchers' hypotheses about events as widely varied as the destruction of Sodom and Gomorrah and the death of King Arthur (said by some to relate to a catastrophic comet impact) suffer similar problems of time and space. Efforts to connect myths with comet or meteorite impacts have met with skepticism. Repeated, undetected big impacts in human time "contradict everything we know about the rate of impacts on Earth, and the inventory of what's out there now, and their dynamics," says David Morrison of NASA's Ames Research Center in Mountain View, California, head of the global Near Earth Object Working Group, which tracks celestial objects that might endanger Earth.

The pendulum may have swung too far in favor of accepting myths, says social anthropologist Benny Peiser of Liverpool John Moores University in the U.K., who runs the Cambridge Conference Network, an Internet clearinghouse for catastrophist theories. Now that more people are willing to listen, he says, too many scientists are invoking myth "left, right, and center to explain everything." In a paper at a late-October workshop on natural catastrophes in the ancient Mediterranean, he asserts that no major myths have yet met scientific standards, although he does credit some regional ones, such as the Pacific Northwest earthquakes. "That's not all bad," he says. "This is all so new, you expect more speculation than hard evidence. The refinements can come later."

From his perspective as a storyteller, James Rasmussen, the record-store owner, also expresses reservations about how much myths can reveal. When he and Ludwin reached the spot where the *a'yahos* boulder was supposed to be, it was gone. In its place was a big wooden chair in front of someone's beach house. "Maybe it's been hauled away," said Ludwin. "Maybe the tide buried it in the sand," said Rasmussen. They poked around for a while among the foam cups, logs, and newspapers littering the beach and finally gave up. "Maybe some things show themselves for a while, and we get a little understanding," said Rasmussen. "Then they go away again, and they don't want to be found."

–KEVIN KRAJICK

Kevin Krajick is the author of *Barren Lands: An Epic Search for Diamonds in the North American Arctic*.



Fire in the sky. A mythic battle between the Hawaiian volcano goddess Pele and the half-pig, half-human Kamapua represents simultaneous appearance of Halley's Comet in 1301 and the biggest known eruption of Kilauea volcano, researchers say.

CREDIT: DIETR C. I VAREZ

Molecular Biology

P-Bodies Mark the Spot for Controlling Protein Production

Serving as sites for RNA degradation and storage puts the tiny speckles at the heart of the cell's machinery for regulating protein synthesis

In the past few months, tiny cellular structures with the unglamorous name P-bodies have captured cell biologists' attention. Mere specks in the cytoplasm, they have been shown to play key roles in regulating one of the cell's most important activities, protein synthesis.

Efforts to understand how cells control the production of their many proteins have typically focused on the first step in the process: the reading of genes to create the messenger RNAs (mRNAs) that in turn direct the actual protein synthesis. Researchers had thought that once mRNAs had done their job, enzymes in the cytoplasm simply broke them down. About 2 years ago, however, several groups showed that much of this degradation occurs in P-bodies—or, as they are sometimes known, GW or Dcp bodies. Now, a flurry of results indicates that the particles are much more than just mRNA chop shops. They appear to play a more dynamic role, serving as routing stations that can temporarily store mRNAs before sending them out to be translated into the proteins that cells need.

Still more recent evidence has linked P-bodies to another exploding area of biology, RNA interference (RNAi). In this phenomenon, which many companies are seeking to exploit to treat diseases, short segments of double-stranded RNA shut off gene expression by directing the destruction of the corresponding mRNAs. This RNA breakdown, which helps cells fight off viruses and genetic damage, may also take place in P-bodies.

Given their apparently broad role in controlling mRNAs, it is perhaps not surprising that there are hints that P-bodies are involved in disease, including cancer and certain autoimmune conditions. As Paul Anderson of Harvard's Brigham and Women's Hospital in Boston, Massachusetts, points out, "regulation of mRNA translation is a very fundamental process with profound implications for cell metabolism."

P-body origins

The path that led to the discovery of P-bodies began about 10 years ago when researchers were studying a key step in mRNA degradation. Before these messengers can be broken down, cells have to knock off a so-called cap, consisting of methylyated guanosine, attached to the mRNA's beginning end. In the late 1990s, Roy Parker's team at the University of Arizona in Tucson cloned the yeast

genes for the decapping enzymes (Dcp 1 and -2) as well as the genes for several proteins that activate the enzymes. Several groups, including those of Bertrand Seraphin at the CNRS Center of Molecular Genetics in Gif sur Yvette, France, Michael Kiledjian at Rutgers University in Piscataway, New Jersey, and Jens Lykke-Andersen at the University of Colorado, Boulder, soon showed that mammalian cells make similar proteins.

Examination of the distribution of the decapping enzymes and other proteins by these researchers and by Reinhardt Luhrmann and colleagues at the Max Planck Institute of Biophysical Chemistry in Gottingen, Germany, revealed that the proteins are concentrated in discrete foci—the P- or Dcp bodies—along with other enzymes involved in mRNA breakdown. This suggested that the particles could be a site of mRNA decapping and breakdown, a supposition confirmed by further experiments. For example, inhibiting the enzyme that degrades decapped mRNAs leads to accumulation of mRNA in P-bodies, which increase in size as a result.

The demonstration that P-bodies are the cell's mRNA destruction sites has since led to a growing appreciation of their diverse roles in the cell. They may, for example, help cells protect themselves against certain stresses. Infection by viruses or exposure to insults such as heat causes cells to turn down their protein synthesis by sequestering their mRNAs in granules. Recent work by Anderson, Nancy Kedersha, also at Brigham and Women's, and their colleagues has shown that these stress granules and P-bodies come into contact with one another and carry some of the same mRNAs. Anderson speculates that the interaction may facilitate what he calls an "RNA triage," with some being maintained in the stress granules while others are shuttled to P-bodies for destruction.

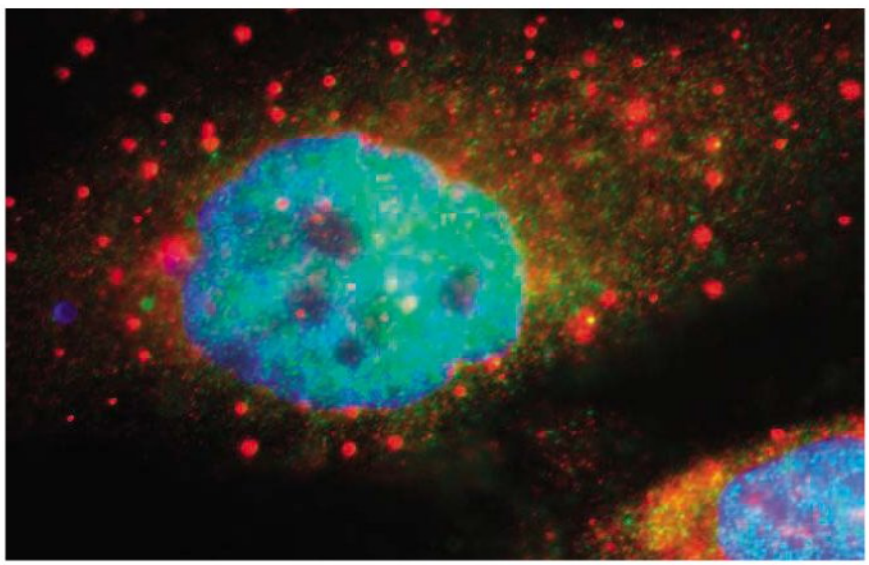
A greater understanding of P-body function may also resolve a lingering mystery about RNA interference: Where does the mRNA degradation it elicits take place? About 6 months ago, George Sen and Helen Blau at Stanford University School of Medicine and Parker, working with Gregory Hannon at Cold Spring Harbor Laboratory on New York's Long Island, and colleagues found that the proteins Argonaut 1 and -2, which are key components of the RNAi machinery (known as RISC), concentrate in P-bodies, implicating the particles as the site of degradation.

The work is also shedding light on a related phenomenon in which so-called microRNAs (miRNAs), which can be produced naturally by cells, repress the translation of mRNAs into proteins. Although this involves the RISC machinery, it apparently does not result in mRNA degradation. The Parker-Hannon team, as well as that of Witold Filipowicz at the Friedrich Miescher Institute for Biomedical Research in Basel, Switzerland, found that mRNAs subject to miRNA repression accumulate in P-bodies in a manner dependent on miRNA function. This suggests that RISC proteins direct the mRNAs to the P-bodies, possibly for storage. Such an idea is consistent with other findings suggesting that the particles do not just degrade mRNAs but also temporarily sequester them away from the translation machinery. Parker and his colleagues reported online in *Science* on 1 September that mRNAs can move out of P-bodies and move to the polysomes, where protein synthesis occurs. Parker says he noticed early on that P-bodies resemble the granules that store the maternal mRNAs that function in very early embryo development. "Even in 2003, we speculated that they [P-bodies] are not just dead ends," he says.

The storage of mRNAs in P-bodies could help regulate embryonic development. In the 19 August issue of *Molecular Cell*, Min Han and his colleagues at the University of Colorado, Boulder, report that a worm developmental control gene encodes a protein that localizes to P-bodies and interacts with the same Argonaut molecules involved in regulation by miRNAs.

The structures may even play a direct role in regulating protein synthesis. Working with yeast, Parker and Jeff Coller, also at Arizona, have shown that cells lacking two P-body proteins (Dhh1p and Pat1) can no longer turn off protein translation in conditions in which it would normally be repressed. P-body concentrations declined dramatically in those cells, the researchers reported in the 23 September issue of *Cell*.

Conversely, translation was repressed in cells engineered to have an overabundance of the two proteins—to the point where the cells could no longer grow. These cells had huge P-bodies. Parker proposes that there is a balance in the cell between two competing events: translation at the polysomes and P-body formation. The question for the cell, he says, is "can I assemble an initiation complex [for protein synthesis] before the mRNA is dragged off to P-bodies?"



Hot spots. In this human tumor cell, P-bodies (red) surround the nucleus.

Possible connections between P-bodies and disease are beginning to emerge. One came in 2002 from a team including Marvin Fritzler of the University of Calgary in Canada and Edward Chan of the University of Florida, Gainesville, who chanced upon the particles while studying a patient suffering from an autoimmune form of nerve degeneration. Using antibodies prepared from the patient's blood serum, the researchers identified a protein they called GW182 and showed that it localizes to speckles in the cell cytoplasm.

The speckles turned out not to be any of the cell's known particulate structures, Fritzler says, so the researchers dubbed them GW bodies. But the work on P-bodies, which was emerging at the time, caught the attention of Fritzler and Chan, and they joined forces with Seraphin to show that the two cellular bodies were in fact identical.

In addition, Seraphin and his colleagues have found that human P-bodies contain a protein called RCK that may help drive cancer development. Researchers have found that its concentration, along with the number of P-bodies, is elevated in various cancers, including breast cancer. A disease link for P-bodies is "a possibility we can't ignore," Chan says, "but further work is necessary to pin it down."

—JEAN MARX

CREDITS: OLIN BLOOM/UNIVERSITY OF ARIZONA

A New Cancer Player Takes the Stage

MicroRNAs are being implicated in various human cancers, and scientists are trying to sort out just how culpable they are

For Frank Slack, the story began when his worms exploded through their vulvas.

It was 1997, and the developmental biologist, now at Yale, had been tinkering with microRNAs (miRNAs), tiny RNA molecules that regulate gene expression. Slack is a worm man, and in his wriggly subjects he had deleted the gene for just one of the 120 known worm miRNAs.

The developing animals' stem cells failed to morph into specialized cells as they normally do and instead kept dividing. "The worms looked extended, weirdly floppy; they kind of looked uncoordinated," he says. The vulvas didn't develop properly and ruptured. A worm skeleton is under hydrostatic pressure, and with the rupture, "the animals burst through," an experience that killed roughly half of them.

Sorry fate. A worm without a microRNA bursts through its vulva (arrow, *inset*); replacing the microRNA keeps the worm intact.

When Slack probed the underlying genetics, he uncovered something tantalizing that linked these unfortunate animals to human biology. Deletion of this miRNA, called let-7, prompted overexpression of a gene, *Ras*, that's strongly associated with many cancers. In other words, when let-7 is expressed normally, it seemed, it blunts *Ras*. Since Slack's find, the let-7–*Ras* story has unfolded rapidly, one of a growing bundle of strands tying miRNAs to cancer.

More than a dozen papers have shown that miRNAs are expressed differently in cancerous tissue. Braided together, the latest miRNA discoveries suggest potentially vast roles for the tiny molecules in malignancy; they have also sparked spirited debate over whether miRNAs are driving cancer or are simply a marker of it. Either way, the nascent field could eventually assist doctors in diagnosis, prognosis, and possibly treatment. Last week, a paper in the *New England Journal of Medicine* (*NEJM*) reported that 13 miRNAs form a signature associated with prognosis and disease progression in patients with chronic lymphocytic leukemia (CLL), a cancer of blood.

"There is a whole other world out there, which I don't think we know anything about," says Phillip Sharp of the Massachusetts Institute of Technology (MIT) in Cambridge, who has studied small RNA molecules for years and is examining their influence on tumors.

Cancer connections

With rare exceptions, it's far from clear which genes the miRNAs are targeting, how many miRNAs are involved in cancer—and how they're involved—and what governs miRNA behavior. Uncertainties aside, however, Sharp and others are not surprised that miRNAs are being implicated. Many of the dozen or so animal miRNAs of known function play a big role in early development. In fruit flies, some miRNAs govern apoptosis, or cell death; in worms, as Slack witnessed to dramatic effect, they control cell differentiation. Both processes, like many others in development, are critical components of tumor formation and spread. "There were these clues," says Joshua Mendell, a geneticist and molecular biologist at Johns Hopkins University in Baltimore, Maryland, who set up his own lab last year and began exploring the miRNA-cancer connection.

Mendell chose to focus on a proto-oncogene called *c-Myc*; proto-oncogenes (*Ras* is another) are often highly expressed in cancerous tissue and implicated in initiating malignancy. "Even though *Myc* has been studied for several decades, [it's] still not fully understood how it causes tumors," says Mendell. Examining a human cell line in which *c-Myc* expression could be manipulated, Mendell and his colleagues found that when expressed, *c-Myc* activates a cluster of six miRNAs. More important, another gene that's both a target of *c-Myc* and drives cell division damps down its expression when two miRNAs in Mendell's cluster are active. That suggested that this miRNA pair could control the balance of cell death and proliferation driven by *c-Myc*.

While Mendell and his team were sifting through their cell samples, a cell biologist at the University of North Carolina, Chapel Hill, was studying how miRNAs might drive lymphoma. Unaware of Mendell's findings, Scott Hammond hit on seven relevant miRNAs in human cancer cells; the cluster was nearly identical to Mendell's list. "We both kind of came to the same group of miRNAs," says Hammond.

But Hammond recognized a problem. Cancerous cells contain abundant abnormalities, many a result of cancer rather than a cause. Hammond didn't know into which category his miRNAs, which were strikingly overabundant in cancer tissue compared with normal tissue, fell.

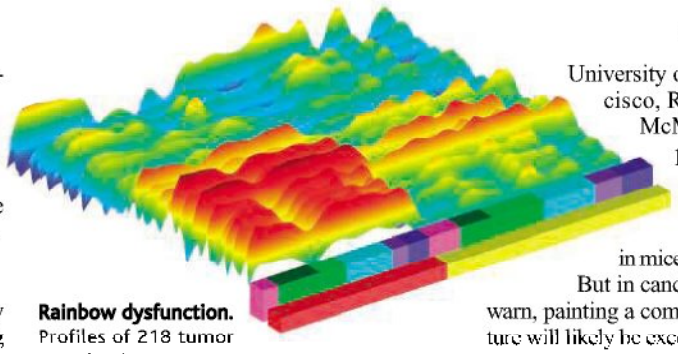
Teaming up with Greg Hannon at Cold Spring Harbor Laboratory in New York, the pair and colleagues forced overexpression of six of the miRNAs together in 14 mice predisposed to a form of lymphoma. Cancer accelerated dramatically. After 100 days, all the treated mice had cancer, compared with about a quarter of controls. The work is "precedent setting," says Sharp, one of the first times miRNAs have been shown to spark cancer. If other miRNAs are found to target either proto-oncogenes, which can trigger cancer, or tumor suppressors, which squelch it, that would further incriminate them.

Hammond and Hannon's work appeared in *Nature* this past June, along with the studies from Mendell's lab and from Todd Golub of Harvard Medical School and the Dana-Farber Cancer Institute in Boston and his colleagues. Golub's research used expression of miRNAs to classify different types of tumors.

But the Hammond-Hannon work remains the exception; nearly all the research implicating miRNAs in cancer does so indirectly. One of the only other studies showing potential causality comes from Carlo Croce of Ohio State University in Columbus, the first cancer

CREDIT: S. JACK/YALE UNIVERSITY

geneticist to publish on miRNAs. In September, Croce reported that in patients with CLL, the loss of two miRNAs boosts expression of a gene promoting cell survival. The gene is believed to help drive the leukemia in its earliest stages. Without the miRNAs that mediate it, leukemia can set in.



Rainbow dysfunction. Profiles of 218 tumor samples from various cancers show miRNA expression as colored "hills."

Elusive quarry

In retrospect, says Harvard RNA expert Gary Ruvkun, given the broad roles being assigned to miRNAs in cancer, it's amazing that cancer geneticists so thoroughly missed miRNAs. "I just find it hard to believe that the cancer people were that lame," says Ruvkun, who is just now starting to back a miRNA-cancer connection.

"We share a collective guilt as a community," agrees René Bernards, a cancer geneticist at the Netherlands Cancer Institute in Amsterdam who is not studying miRNAs. With a laugh, he recalls his graduate school days, when he tossed "anything small, degraded, uninteresting" in the trash. At the time, miRNAs fell squarely in that category. Furthermore, miRNAs are generated by genes that don't produce proteins—long derided as "junk" DNA.

Indeed, Croce, now a consummate miRNA fan, admits being dragged into the field unwittingly. Ten years ago, he grew convinced that a CLL tumor-suppressor gene was nestled in a certain stretch of DNA—but he couldn't spot it. Baffled and stubbornly determined, Croce turned to colleagues in the CLL field, who handed over additional leukemia samples to scour. Only when Croce stopped looking for a coding gene 3 years ago did he settle on the two miRNA genes he's been studying ever since.

With the outlines of a miRNA-cancer connection taking shape, researchers are now beginning to tackle some of the toughest questions. Perhaps the most vexing involves finding miRNA targets. Like other types of small RNA molecules, miRNAs influence genes with a similar sequence—but the match need not be exact, making the targets maddeningly hard to pin down.

No experiment "can hand you a target on a silver plate," says Nikolaus Rajewsky, a biologist and mathematician at New York University. These days, says Rajewsky, the best target-finding melds two tactics. The more traditional compares putative miRNA targets in mammals with known targets for the same miRNA in other species. The other calls for over- or underexpressing a miRNA, then running microarray studies to spot affected genes. But "the computational approaches are still evolving; the experimental approaches are labor-intensive," says Victor Ambros, a geneticist at Dartmouth Medical School in Hanover, New Hampshire. "What we're not sure about is how many targets we're missing."

Several labs are conducting massive miRNA knockout studies to delineate the targets and functions of individual miRNAs. At the University of California, San Francisco, RNA biologist Michael McManus is leading a six-person mouse miRNA consortium; it plans to delete each of the 350 known miRNAs in mice, one at a time.

But in cancer especially, biologists warn, painting a comprehensive miRNA picture will likely be exceedingly complex. When miRNAs "get overexpressed or underexpressed or deleted, lots of things can happen," says Tyler Jacks, director of MIT's Center for Cancer Research. "And trying to figure out exactly which of those things is contributing to tumorigenesis or prognosis or what have you" calls for "a lot of detective work."

Nor is it clear what prompts miRNAs to misbehave in the first place. "We'd really like to know," says Slack, who theorizes that mutations in miRNAs could be at fault, as could defects in transcription factors, proteins that control gene expression. Croce has found two leukemia patients born with the miRNA mutations implicated in CLL.

Looking ahead

Given all the unknowns, miRNAs are a long way from the clinic. But some drug companies are dabbling in them nonetheless. Jan Weiler, a chemist at Novartis in Basel, Switzerland, has been studying the role of miRNAs in disease for 2 years. (In addition to cancer, the molecules are tentatively linked to neurological disorders and diabetes.) "It's a lot of speculation, a lot of hope," says Weiler, who envisions perhaps delivering miRNAs to patients lacking them. "If we don't look at it now, we're probably too late," he says, while acknowledging the risk that "maybe … in 3 years' time, the whole thing is dropped."

If therapeutics remain distant, diagnostics are closer to reality. Croce co-authored last week's *NEJM* paper that reported on a 13-miRNA signature in CLL. His group also found that among 94 CLL patients, many of those lacking Croce's original two miRNAs have a milder form of CLL, whereas most with the two functioning miRNAs suffer a more aggressive form. "It looks like CLL is not one disease but two," he says, and the distinction could be useful in diagnosing and treating the leukemia.

Other cancers, too, are being eyed as harboring miRNA culprits. One of the very first miRNAs tied to cancer—let-7 with its exploding worms—was last year found to be lacking in lung cancer tissue taken from patients in Japan. Those with the lowest levels fared the worst—suggesting once again that flawed miRNA expression bodes poorly for one's health.

—JENNIFER COUZIN

Under the influence. Chronic lymphocytic leukemia cells (*above*) appear to be driven by miRNAs.

CREDITS (TOP TO BOTTOM): G. GETZ, J. LAND T. GOLUB; D. AMAR/PHOTO RESEARCHERS INC.

Synthetic Biology Remakes Small Genomes

Researchers are taking the first steps toward realizing the goal of building chromosomes by wholesale remodeling of organisms' genomes

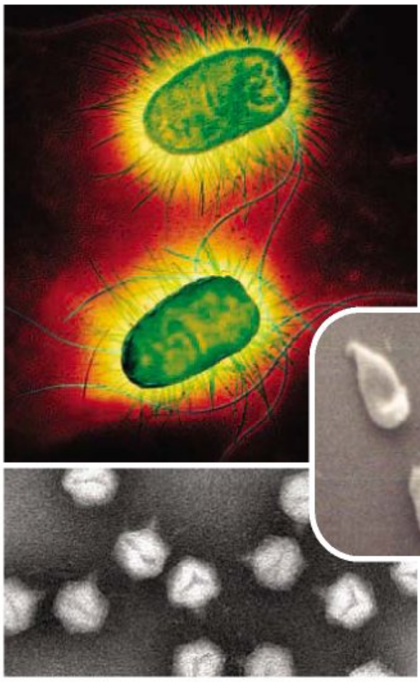
Hilton Head, South Carolina—People just can't leave nature alone. They have long stopped mighty rivers with dams, they are now breeding seedless watermelons, and they soon hope to customize microbes. Researchers from civil engineers to molecular biologists are developing ways to mold genomes like a potter does clay. These efforts to remake bacterial and viral DNA go far beyond adding or deleting a gene or two. Scientists are reducing, stretching, and recreating chromosomes as they lay the foundation for the emerging field of synthetic biology. "What we are most excited about are useful things we can make by messing around with the whole genome," says George Church, a technologist at Harvard University in Cambridge, Massachusetts.

Through their genome manipulations, synthetic biologists expect to learn more about how microorganisms function and also harness them to make complex proteins, get rid of toxic wastes, or carry out tasks not yet envisioned. Some of this new field's progress was on display at a genome meeting last month.* "You sensed a lot of excitement and stirring," says Ari Patrinos, chief of genome research at the U.S. Department of Energy. "It reminds me of the very early days of the Human Genome Project."

At this point, however, the field is more talk than reality, says J. Craig Venter, president of the J. Craig Venter Institute in Rockville, Maryland. "There's not a lot of data yet." It's difficult to separate the hype about synthetic biology from the hard results, agrees Patrinos. "This is the frontier" of biology, he notes.

Some of the hard results discussed at the meeting came from geneticist Frederick Blattner of the University of Wisconsin, Madison, who has gradually been shrinking the genome of *Escherichia coli*. The altered bacterium hardly notices, and it may offer advantages for genetic engineering, he reported.

Blattner began trimming the microbe's genome after sequencing various *E. coli* strains. He found that although the strains had 3.7 million bases in common, each also had about another million bases—cordoned off in specific "islands" of DNA—unique to each strain. His group has been deleting these genetic islands and other bits of DNA one by one, checking that the bacteria survive despite each loss. They perform these excisions using the natural process of homologous recombination. For example, they introduce into bacteria a stretch of DNA containing the sequences on either side of an island. A small number of the microbes will then swap out their similar stretch of DNA for the synthetic island-free version. The process is "scarless," as no extra DNA is left behind.




Designer bugs. *E. coli* (*above*), mycoplasma (*inset*), and bacterial virus (*lower*) studies are leading to customized chromosomes.

CREDITS (TOP TO BOTTOM): OLIVER MECKES/NICOLE OTTAWA/PHOTO RESEARCHERS INC.; KENNETH BOTT/UNIV. OF NORTH CAROLINA, CHAPEL HILL; DEAN SCHOLL/NIH

So far, the group has made 43 such deletions, whittling the core *E. coli* genome to less than 4 million bases and 3500 genes. That's far fewer than the 4444 genes now known to exist in the *E. coli* sequence. The researchers plan to trim even more, cutting another 30 islands. "By then, we think we will have removed most of the nonessential material," Blattner said.

With its lean bacterial chromosome, the streamlined *E. coli* strain created by Blattner's group is 10 times better at absorbing new genes than one of the strains commonly used in genetic engineering. Now, "he can take this reduced genome and begin to add in [genes for] important industrial or pharmaceutical pathways," says Hamilton Smith, a molecular biologist at the Venter Institute. Moreover, notes Blattner, his new strain should be more resistant to certain undesirable genetic changes because it lacks the DNA islands, which tend to hop around the genome creating mutations.

Pump up the genome

In contrast to those who would shrink microbial chromosomes, Drew Endy of the Massachusetts Institute of Technology (MIT) in Cambridge has been expanding one. A civil engineer, Endy is one of the most visible—and controversial—spokespersons for the synthetic biology field. He runs a yearly contest in synthetic biology that has grown beyond MIT to include international teams (*Science*, 9 January 2004, p. 158). One of the most innovative entries thus far has been a bacterial camera, in which researchers endowed bacteria with genes for light-sensing proteins and other components for generating an image on culture media.

On his lab's synthetic biology Web site, Endy has set up a virtual bulletin board of research ideas, results, and protocols in the field; it draws 15,000 visitors a day. Some of his peers privately complain that Endy is a larger-than-life self-promoter—he's got his own synthetic biology company, gives scores of talks worldwide each year, and has helped create an upcoming comic strip with a main character called Device Dude who is a synthetic biologist. Others argue that he's driving the field forward. "He's injecting a lot of rigor in a field that is still somewhat soft," says Patrinos.

At the meeting, Endy described his lab's unusual work on T7, a virus that infects bacteria. He had been bothered by genes in T7's genome that were embedded or partially embedded in other genes and therefore shared some of the same DNA, as they complicated his ability to predict how infection and the resulting incorporation of viral DNA into the host genome are affected by different host environments. His model treated all the genes as separate entities and didn't take into consideration what happens if genes overlap. So he and his colleagues pulled apart T7's overlapping genes by inserting an extra copy of the overlap next to the original such that both genes, now separated, still had their full complement of bases.

Worried that they might kill the virus as they pumped up its chromosome, he and his colleagues only added 600 bases to its 40,000-base genome in this initial round of experiments, hoping that removing the overlaps didn't disrupt the genes' regulation or

* Genomes, Medicine, and the Environment 2005, 16–19 October, Hilton Head, South Carolina.

impair their function. The engineered virus was still able to invade bacteria and replicate, according to Endy. "We've demonstrated it's possible to redesign a genome" beyond adding individual genes, he says. Now, he and his colleagues are adding more bases to the T7 genome, testing the limits of this expansion technique.

Making genomes bigger or smaller is just a tiny step in realizing the true potential of synthetic biology. The field needs to move forward on many fronts, says Venter. Synthesizing new chromosomes from scratch, for example, remains a challenge. In one effort in that direction, Smith and his colleagues have for the past few years been knocking out individual genes in *Mycoplasma genitalium*, which has the smallest known genome of a free-living organism (*Science*, 14 February 2003, p. 1006). So far, they've identified about 100 genes, out of nearly 500, that *M. genitalium* can live without.

Their eventual goal is to identify the microbe's essential sequences and then see if they can synthesize and assemble just those sequences and use them to create a living organism by inserting the humanmade chromosome into a cell. Among the many details to be worked out, says Smith, is how to piece together relatively huge sections of DNA. Ideas include using live cells to put together chunks of DNA into a whole mycoplasma chromosome or putting an efficient DNA repair system—such as seen in bacteria resistant to radiation damage—into a test tube to accomplish this task. Then his team must determine how to stick this DNA into a cell and remove the native DNA, without affecting the cell's ability to function.

Ethical and environmental concerns must also be dealt with before synthetic biology fully matures as a field. MIT, the Venter Institute, and the Center for Strategic and International Studies in Washington, D.C., have teamed up to examine issues such as how to keep any new life forms created under control. This effort is funded by a $570,000, 15-month grant from the Alfred P. Sloan Foundation. Some researchers are already exploring strategies to incorporate safeguards. For example, Church and Endy are developing ways to keep synthetic genes from escaping and possibly wreaking havoc. One solution: Alter synthetic genetic codes such that they are incompatible with natural ones because there is a mismatch in the gene's coding for amino acids.

A final issue confronting synthetic biology is cost. The bigger the DNA piece synthesized, the less accurate the sequence and the more expensive it is to get it right. But new technologies are rapidly coming on line, note researchers. "The cost of accurate DNA synthesis and sequencing is plummeting, and as it does, we will see a quantum shift in what people dream of and do," says Church.

–ELIZABETH PENNISI

Education

Forging a Cosmic Connection Between Students and Science

By deploying cosmic-ray detectors at high schools, physicists hope to inspire students and score real scientific discoveries to boot

Twelfth-grader Treasure Sheppard has aspired to become an aerospace engineer since she was 7 years old. But nothing fired the bright and bubbly 17-year-old's passion for science and technology quite like a weeklong visit to the California Institute of Technology (Caltech) in Pasadena, where she and a classmate assembled a detector to snare cosmic rays—subatomic particles zooming in from space. "I was expecting a few lectures" from Caltech physicists, says Sheppard, who attends nearby South Pasadena High School. "But when we got there, they handed us a piece of paper and said, 'These are the instructions.' They had confidence that we could complete the task."

That detector is now part of the California High School Cosmic Ray Observatory (CHICOS), an array of detectors stretching across the roofs of 70 high schools and middle schools in metropolitan Los Angeles. Unlike typical high-school science projects, CHICOS aims to do cutting-edge research by probing the nature of cosmic rays. That prospect thrills Sheppard, who last year tended the two detectors on her school's roof. "CHICOS gave me an opportunity to participate in research," she says, "which some college students can only dream of."

CHICOS is one of several arrays that have sprouted up across North America and Europe. Using salvaged parts, a little newfangled electronic gadgetry, and student labor, particle physicists are outfitting schools from rural Nebraska to downtown Amsterdam with simple, inexpensive cosmic ray detectors. At least six sizable arrays are up and running, and as many more are in the planning. Physicists aim to stimulate teachers and students by bringing real science into the classroom. At the same time, they hope to grab scientific glory on the cheap by discovering phenomena that more-expensive research arrays might miss.

Cosmic rays enable educators to bring science to the students instead of busing the students to visit some distant lab, says Gregory Snow, a physicist at the University of Nebraska, Lincoln, and leader of the Cosmic Ray Observatory Project (CROP), an array with detectors at 26 schools across the state. "Cosmic rays are going through every high school in the world all the time," he says. "That allows you to get people involved in research right where they live and go to school." The National Science Foundation has funded several of the arrays, and the primary goal of the projects is education, says Randal Ruchti, a program officer in experimental particle physics at the foundation. Still, he says, it's possible that "a student could participate in a revolutionary discovery."

To fulfill both their educational and scientific missions, however, the projects must balance the students' need to tinker with the detectors against researchers' need to keep machinery running full-time. And there's no science that can tell physicists how to strike the proper balance.

Finding a niche

Every second, hundreds of cosmic rays pepper every square meter of Earth. If a ray has enough energy when it crashes into the atmosphere, it produces a cascade of particles known as an "extensive air shower." For decades, physicists have studied air showers with detectors arrayed on the ground, using the

CREDIT: SC. IOTANUS/RADBOUD UNIVERSITY OF NIJMEGEN

size and the timing of the signals from the individual detectors to estimate the energy and direction of the cosmic ray.

Since the 1990s, physicists have known that a very few cosmic rays crash into the atmosphere packing as much energy as a large hailstone. No one knows how an individual subatomic particle obtains such tremendous energy or precisely how often one strikes. Professional cosmic ray arrays—most notably the Pierre Auger Observatory, an array of 1600 detectors stretching over 3000 square kilometers currently under construction in Argentina—focus on those questions.

But some physicists hope to build arrays on the cheap by placing detectors on the roofs and grounds of schools—and more than one claims to have had the idea first. The detectors typically consist of sheets of plastic "scintillator," which emit light when penetrated by charged particles. Often, as is the case with CROP and CHICOS, the scintillators are left over from decommissioned professional arrays. For a few thousand dollars, researchers outfit a school with its own miniarray of a few detectors, a Global Positioning System station to tell precisely where each detector is and when it registers a hit, and a computer to collect data and ship it to the researchers via the Internet.

The arrays differ in essential details. For example, the schools in CHICOS are as little as a kilometer apart, so several may register hits from a single large shower. Schools in CROP are separated by hundreds of kilometers, so even a big shower will likely strike only one. Some arrays are more polished and professional than others. For example, physicists build the detectors for the Alberta Large Area Time Coincidence Array (ALTA), which is run by the University of Alberta in Edmonton and has detectors at 15 schools. In contrast, high-school students cobble together the detectors for the Washington Large Area Time Coincidence Array (WALTA), which is run by the University of Washington, Seattle, and has detectors at 11 schools. "Ours is more of a roll-your-own approach," says Jeffrey Wilkes, a particle physicist at the university.

High-school arrays cannot compete toe-to-toe with Auger, says Mark Pearce, a particle physicist at the Royal Institute of Technology in Stockholm, Sweden, and leader of the Stockholm Educational Air Shower Array, an array of detectors at the institute and four secondary schools around the city. But "there are theories that the professional arrays are not designed to test, and certain interesting, well-defined questions that these school arrays might be able to answer," he says. For example, with schools spread over even larger areas, the arrays might test whether cosmic rays arrive in widespread bursts instead of completely at random. That could happen if an iron nucleus from space collided with a photon from the sun and splintered into pieces.

Some researchers hope to carve out a niche by literally looking where Auger cannot. Auger observes only the southern sky, which may differ from the northern sky when viewed in cosmic rays, notes Robert McKeown, a particle physicist at Caltech and leader of CHICOS. "We are the largest array in the Northern Hemisphere," he says, "and if an unusual event occurs in the Northern Hemisphere, we may be able to see it."

Hands-on. Rooftop detectors engage teachers and students in research.

CREDIT: JOHN CATTEDI

Others hope to use high-school arrays to develop new detection techniques. Physicist Helio Takai and colleagues at Brookhaven National Laboratory in Upton, New York, plan to use a high-school array on Long Island to test an antenna that detects radio waves reflected by the charged particles in an air shower. They've dubbed their project Mixed Apparatus for Radio Investigation of Atmospheric Cosmic Rays of High Ionization, or MARIACHI. By comparing readings from the array with those of the antenna, Takai and colleagues hope to show that the low-cost radio technique is effective.

Unanswered questions

Regardless of their specific scientific goals, all the arrays hope to spark students' interest in science. And some students say that the projects have succeeded handsomely. Mark Jeroncic participated in ALTA while he was a student at Edmonton's Holy Trinity High School. Using data collected with his school's detectors, he found a correlation between the rate of cosmic rays and ozone levels in the city. Now in his second year at the University of Alberta, Jeroncic says his experience with ALTA led him to major in physics.

Most physicists recognize that reaping a rich data harvest may conflict with giving students a chance to take the detectors apart and fiddle with them to see how they work. And they disagree about which aspect projects should emphasize. "We think that the real thrill for the students is to be part of a research project, so we've always strived to make this a professional array," says James Pinfold, a physicist at the University of Alberta and leader of ALTA. To that end, ALTA researchers build and install the hardware. "We give the students the data to play with rather than the detector," Pinfold says. ALTA physicists give students smaller scintillator detectors to use in classroom experiments.

But students may feel little connection to the main array if they never get to touch it, says Charles Timmermans, a particle physicist at Raboud University in Nijmegen, Netherlands. Timmermans heads the High-School Project on Astrophysics Research With Cosmics (HiSPARC), an array with 35 miniarrays at schools in Nijmegen, Amsterdam, and other cities. "Small detectors are nice, but you have to give students the feeling that the array on the roof is theirs," he says. "If you don't give them a chance to work and play with it, I think that after the first generation of students, that feeling will fade pretty fast." Timmermans favors designating a week each year to let students rebuild the detectors.

Ultimately, it may be hard to predict what will inspire any individual student. Loran de Vries, who attended the Amsterdams Lyceum and participated in HiSPARC, says he was most impressed by the inability of physicists to answer basic questions about the origins of high-energy cosmic rays. "I saw with my own eyes that in this subject, most of these things are not known, and I found that fascinating," says De Vries, currently a second-year student at the University of Amsterdam. Thanks in part to his experience with HiSPARC, De Vries wants to become a high-school physics teacher. Perhaps when the time comes, he'll be able to answer those questions for his own students.

–ADRIAN CHO

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RANDOM SAMPLES

Edited by Constance Holden

Raising the Dead

Long thought extinct, a historically important Belgian grass has been resurrected from the vaults of a seed bank.

Earlier this year, David Aplin of the National Botanic Garden of Belgium was rummaging through the garden's collections in preparation for a meeting of the recently formed European Native Seed Conservation Network. He came upon long-forgotten packets of seeds from *Bromus bromoideus*—the "Brome of the Ardennes"—a grass species that had been wiped out in the wild 70 years ago.

A modern drawing of *Bromus* by Omer Van De Kerckhove.

Bromus is the only plant ever found to be unique to Belgium, where it flourished in the rolling, chalky meadows of the Ardennes. Its image was embossed on the cover of several 19th century books on Belgian flora. But changes in land tilling led to its disappearance. Botanists, more concerned with exotic varieties than native plants, "took their eye off the ball" and failed to keep the species going, Aplin says.

Now botanists have succeeded in getting the *Bromus* seeds to germinate, and there are little green shoots from them growing in both Belgium and England.

Tracking Mini-Fauna

From the home of precision watch works now come radiotransmitters tiny enough to track insects.

Behavioral ecologist Beat Naef-Daenzer of the Swiss Ornithological Institute in Sempach and his colleagues wanted to study young barn swallows preparing to leave their nests, but no transmitter on the market fit the job. So they created their own from the smallest components available, coming up with a 200-milligram instrument capable of broadcasting over a 2-kilometer range for 3 weeks.

Owl butterfly with transmitter on its back.

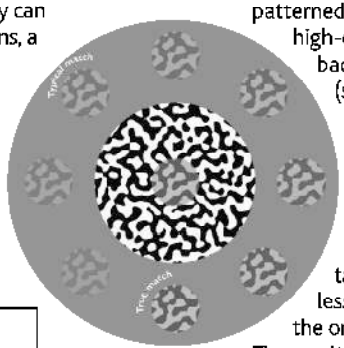
The researchers have now moved beyond swallows and are field-testing the instrument on owl butterflies, which weigh about 2 grams. Most animal species weigh less than 20 grams, notes Naef-Daenzer, and population movements of many are "virtually unknown because individuals cannot be tracked over more than a few minutes." He says minitransmitters could help track little creatures such as tree frogs, African locusts, or Europe's endangered aquatic warbler. The group, whose report appears in the 1 November *Journal of Experimental Biology*, is looking into smaller power sources, such as ultrathin polymer photovoltaic cells.

"Half of the world's birds are too small to use traditional tags for. This opens up a large set of species to do that work with," says ornithologist David Winkler of Cornell University.

Spotting Illusions

People with schizophrenia can't always distinguish real from unreal, but they can see right through some visual illusions, a new study shows.

Schizophrenia seems to include an inability to process the context of things, from social interactions to metaphoric language, explains psychologist Steven Dakin of University College London's Institute of Ophthalmology. So, says Dakin, "we wondered whether that would affect their vision as well."



Dakin and his team showed 15 schizophrenic subjects and 20 controls a shaded, patterned disk against a high-contrast background (see illustration). The subjects were then shown a "reference patch" and had to assess whether it contained more or less contrast than the original image. The results were startling: 12 of the 15 schizophrenic observers were more accurate than the most-accurate member of the control group.

"The illusion's pretty substantial," Dakin says, but "the schizophrenics were almost completely immune to its effect." "We're hoping [the study] might be a step toward more objective diagnostics," he adds.

Psychiatrist William Phillips of the University of Stirling in the U.K. calls the findings "very important." He says "the weakened effects of context" that people with schizophrenia demonstrate may apply across the board in many domains of cognition and perception.

Scope's On

Scientists are finally getting the payoff for disrupting all those red squirrels back in 1996 when construction began on the Large Binocular Telescope (LBT) on Mount Graham in Arizona. Last week, the LBT transmitted its first light image, a spiral galaxy in the Andromeda constellation 24 million light-years away.

The $120 million LBT, the world's most advanced optical telescope, will be able to peer all the way back to the beginning of time—15 billion light-years—with its two massive 8.4-meter mirrors. The scope is to be fully operational by fall next year.

CREDITS (TOP TO BOTTOM): BEAT NAEF-DAENZER; NATIONAL BOTANIC GARDEN OF BELGIUM; STEVEN DAKIN; LBTO/LBTB CONSORTIUM/R. GENDLER

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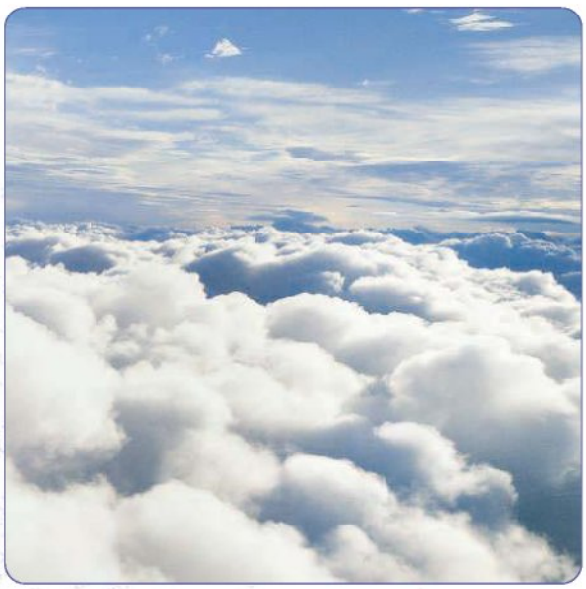

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Edited by Yudhijit Bhattacharjee

JOBS

A long commute. Biochemist Joan Massagué has found a way to serve his native Spain while remaining in the United States. Last month, the 52-year-old head of the cancer biology and genetics program at the Memorial Sloan-Kettering Cancer Center

in New York City (MSKCC) became adjunct director of the Barcelona Biomedical Research Institute (BBRI).

Massagué will work with his former Ph.D. mentor and BBRI director Joan Guinovart to design research programs, recruit new investigators, and formulate policies at the new $20 million institute, which is being funded by the Catalan government and the University of Barcelona. He will also supervise a lab devoted to metastasis biology.

Under an agreement approved by MSKCC, Massagué says he will travel to Barcelona for a few days once every 2 months. He says his appointment is "designed to foster the development of biomedical research at BBRI" without hurting his activities at MSKCC. The arrangement will benefit both institutions, he says; postdocs and graduate students from his Barcelona lab will be able to spend time at MSKCC for short work visits financed entirely by BBRI.

Digging logically. The United Kingdom's biggest center for archaeology research and teaching has a new director. On 1 October, Stephen Shennan, an archaeologist known for applying Darwinian theory to cultural evolution, took over the helm of the Institute of Archaeology at University College London from retiring chief Peter Ucko. The institute, with more than 70 faculty members and nearly 500 students, has trained many of Britain's leading archaeologists.

Shennan came to the institute in 1996 after a 20-year career at the University of Southampton. Insiders say he is an excellent choice, even if his approach puts him somewhat out of step with a tendency among British archaeologists to eschew hypothesis testing. "He has rejuvenated archaeology with ideas and quantitative methods from evolutionary theory," says Rob Boyd, an anthropologist at the University of California, Los Angeles.

As head of the institute, Shennan says he plans to "foster a rigorous approach to understanding the past."

Same trick, new trade. After helping establish a medical genomics laboratory at Rockefeller University in New York City, computational biologist Terry Gaasterland is moving west to create a center that will span marine, comparative, and environmental genomics.

As director of the just-launched Scripps Genome Center at the Scripps Institution of Oceanography in San Diego, California, Gaasterland will lead an effort to apply software tools to make genome comparisons between animals as different as humans and sea squirts and to study marine diversity. "The only way that you can understand that data is through bioinformatics," says microbiologist Mitch Sogin of the Marine Biological

Laboratory in Woods Hole, Massachusetts. "Terry is in the right place."

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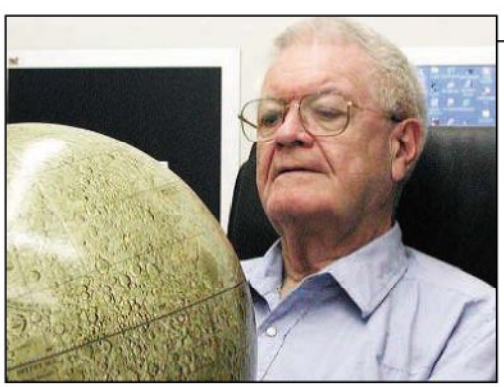
THEY SAID IT

"Now you can all be insiders and say Vuh-NEE-ver [Bush]."

—Former MIT president Charles Vest last month in Washington, D.C., asking fellow members of the Department of Education's newly formed panel on higher education to correct their pronunciation of the legendary science administrator's name.



DEATHS

Shining star. Astrophysicist Alastair Cameron, who was one of the first to suggest that elements form inside the hearts of stars, died in Tucson, Arizona, on 3 October. He was 80.

Cameron spent 26 years at Harvard University, conducting research on astrophysics and planetary sciences, and chaired the Space Science Board of the National Academy of Sciences from 1976 to 1982. He was most recently at the Lunar and Planetary Laboratory at the University of Arizona in Tucson.

Cameron was known for breaking down barriers between disciplines, says astrophysicist W. David Arnett, also of the University of Arizona. "He changed the direction of space and planetary science by [his] example," Arnett says.

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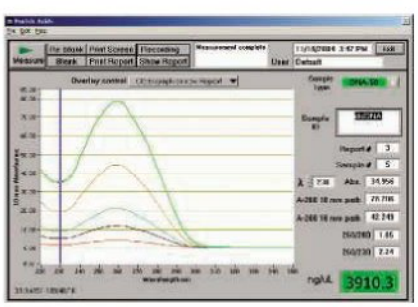

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University Investment in Drug Discovery

ALTHOUGH YOUR SPECIAL SECTION ON DRUG Discovery (29 July, pp. 721–735) highlighted important contributions from academia, it did not recognize an increasingly relevant but underappreciated and underutilized role for academic research in drug discovery.

Universities invest many millions in basic research that exposes disease mechanisms and therefore unearths new targets. Yet few have invested in the relatively modest infrastructure required to put their discoveries to the test. As a result, many promising targets gather dust on the university shelf. This need not be the case. Developing appropriate assays, screening modest-sized compound libraries, using medical chemistry to further develop leads, and conducting preliminary tests in animal models are functions well suited to academia. Academic researchers often have the best understanding of individual targets, routinely design and refine in vitro assays, and have ready access to and experience with the most appropriate animal models.

> "Universities invest many millions in basic research that exposes disease mechanisms... [y]et few have invested in the relatively modest infrastructure required to put their discoveries to the test."
>
> –IVINSON

The pharmaceutical industry (and, to a lesser extent, biotech) look at drug discovery ideas emanating from academic research as too risky and early in development to warrant significant investment. This risk aversion is in large part a reflection of the economic climate and the changing winds of drug-discovery received wisdom. To bring these ideas to a stage where pharma will look at them more carefully, we can and should advance them through at least the first stages of drug discovery. Demonstrating a credible mechanism and target, proprietary lead compounds, and preliminary in vivo efficacy will be enough to bring some of our industry colleagues back to the table. But this will only happen when academics stop treating drug discovery as the intellectually inferior domain of the commercial sector and start seeing it as the natural development of their research.

ADRIAN J. IVINSON

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A Place at the Pharma Table for Women?

THE ARTICLE "IT'S STILL A MAN'S WORLD AT THE top of big pharma research" (J. Mervis, Special Section on Drug Discovery, News, 29 July, p. 724) resonated with me. As a scientist in Merck R&D in the 1990s, it was clear to me that women did not have a place at the decision-making table. As years of diversity committees, on-site day care, mentoring programs, coaching, and other HR efforts rolled by, many talented women figured out that their only career path at Merck R&D was out the door.

Merck, and I suspect many other "big pharma" companies, are feeling the effects of having some of their best talent leaving and taking their brainpower elsewhere. We have started companies, taken senior positions in biotechnology firms, and become leaders in government and academia. Perhaps this brain drain of talented women has exacerbated the problem of the empty product pipelines of big pharma.

The men in charge of R&D tend to promote and recruit other men with whom they feel the most comfortable and ignore talented women. Until they are forced by progressive senior executives to include, in significant numbers, women in their club, they will not change.

LINDA RHODES

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Costs and Benefits of Regulating Mercury

MERCURY IS KNOWN TO HAVE DETRIMENTAL effects on human health (*1*), so it is surprising to read that it may not be worthwhile to regulate mercury releases from U.S. power plants ("Regulating mercury: what's at stake?", T. Gayer, R. W. Hahn, Letters, 8 July, p. 244). Although there is legitimate debate about the cost of implementation and the choice of emission reduction approach, we feel that the estimated benefits of emission reduction of $100 million accrued over 15 years have been grossly understated by Gayer and Hahn.

Their proposed benefit was based on a study of willingness-to-pay for chelation therapy to reduce lead in children. However, lowering levels of lead by chelation has not been demonstrated to improve cognition (*2*). Similarly, although chelation therapy may remove methyl- and ethylmercury, it cannot reverse central nervous system damage (*3*), implying that prenatal mercury exposure leads to lifelong lost benefits, irrespective of money spent on removing the causal agent from the body after the damage has been done.

Thus, an approach based on lifelong losses in income better estimates the benefits of reducing mercury emissions (*4*). This approach attributes subsequent losses in lifelong earnings as a result of lower IQ to the loss in a child's IQ from prenatal methylmercury exposure. The estimated lifelong losses in income for all U.S. children affected in the year 2000 was $1.3 billion per year (range: $0.1 to $6.5 billion), which would lead to a $15.9-billion loss in income (range: $1.2 to $79.9 billion, discounted at a rate of 3% per annum) over the 15-year period considered by Gayer and Hahn. Therefore, by only considering the loss of earnings due to exposure to mercury generated by U.S. power plants, lowering prenatal exposure by reducing emissions may have considerable economic benefits, likely exceeding the estimated costs of $4 billion to $19 billion.

DIRK ZELLER AND SHAWN BOOTH

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Response

IN OUR STUDY, WE ESTIMATED THE COSTS and benefits of the U.S. Environmental Protection Agency's (EPA) power plant mercury regulation. To estimate the benefits of mercury reduction, we considered each link in the pathway, including the reduction of emissions from U.S. power plants; reductions in mercury deposition; reductions of methylmercury in U.S. freshwater and marine fish; reductions of methylmercury consumption from U.S. fish by U.S. resi-

dents; reductions of methylmercury in U.S. women of childbearing age; and IQ improvements in U.S. children. For each link, we used the best available evidence and, if anything, tended to err on the side of overstating benefits. Only at the end did we monetize estimates of IQ improvements, based on a study of parental willingness to pay for IQ increases through chelation.

Zeller and Booth contend that our estimate of the benefits of mercury reduction is "grossly understated" based on their claim that our estimate of the value of an IQ point is flawed. They cite a study by Trasande *et al.* (*1*) claiming that benefits of mercury reduction are $1.3 billion per year. Unfortunately, they are comparing apples with oranges. The $1.3 billion estimate (*1*) is for the benefits of eliminating all U.S. power plant mercury emissions. Zeller and Booth apply this annual measure of complete elimination of power plant mercury emissions to each year from 2005 to 2020. It is incorrect to compare the costs of EPA's regulation that eliminates a fraction of the power plant emissions to the benefits of eliminating all power plant emissions of mercury (which would cost considerably more to achieve).

Zeller and Booth suggest that the monetized benefits we use for IQ may be understated. We agree that the willingness-to-pay numbers for IQ may understate the benefits of IQ. The value of an IQ point suggested by Trasande *et al.* (*1*) is about an order of magnitude greater than our estimate. However, as we noted in our Letter, using their estimate does not change our finding that the costs of the regulation are likely to exceed benefits.

Zeller and Booth's claim of mercury's detrimental effects might be overstated. They cite Grandjean *et al.*'s study (*2*) of the Faroe Islands to support their claim that the detrimental effects of mercury are "known." They do not mention a study of the Seychelles (*3*) that did not find evidence of such a link and a study in New Zealand (*4*) that found mixed evidence. Even Grandjean *et al.* (*2*) found mixed results for the relationship between mercury and IQ scores. Nonetheless, we used conservative estimates of the IQ-mercury relationship even when they are not statistically different from zero.

We think that policy-makers should design regulations for controlling mercury emissions so that expected benefits exceed expected costs. The current approach fails that test.

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Landscape Corridors: Possible Dangers?

THE REPORT "EFFECTS OF LANDSCAPE CORRIDORS on seed dispersal by birds" (1 July, p. 146) by D. J. Levey *et al.* shows that landscape corridors increase the movement of birds between patches of habitat in a fragmented landscape, and that this facilitates the movement of bird-dispersed seeds. Another study, in the same experimental setting, found that corridors increase interpatch insect pollination (*1*). Both studies conclude by emphasizing the conservation value of habitat corridors. However, landscape corridors also facilitate the spread of

invasive alien species (*2*). Although the potential negative effects of habitat connectivity were stated almost two decades ago (*3*), these seem to have been largely ignored in the evaluation of corridors as a conservation tool.

Alien plants with attractive flowers and fruit can hijack generalist pollinators and seed dispersers from indigenous plant species (*4*). By increasing alien propagule pressure, invasive species outcompete and replace local biota (*5*). Indeed, the spread of invaders is often facilitated by corridors, either natural (rivers, coastlines, ridges) or man-made (roads and railways). In this context, it is worth mentioning that all the plants considered in the South Carolina studies [Levey *et al.*; (*1*)] are aliens of concern in parts of the world [*Lantana camara* (*6*), *Rudbeckia hirta* (*7*), *Morella* (=*Myrica*) *cerifera* (*8*)]. Moreover, the Eastern Bluebird (*Sialia sialis*) that dispersed *Morella* seeds is also known to disperse seeds of the alien tree *Sapium sebiferum* in the eastern United States (*9*).

Presently, land managers are advised to build habitat corridors to reduce the effects of habitat fragmentation, but habitat barriers are also built to manage the spread of invasive species (*10*). It is ironic that habitat corridors do not always link the seemingly separate fields of conservation and invasion biology. Both habitat fragmentation and invasive species have resulted in the loss of large sections of biodiversity, and their combined impacts must be better understood. The modeling tools developed in the present study present a useful opportunity for developing a more integrated approach to the evaluation of corridors as a conservation management tool.

ŞERBAN PROCHEŞ, JOHN R. U. WILSON, RUAN VELDTMAN, JESSE M. KALWIJ, DAVID M. RICHARDSON, STEVEN L. CHOWN

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Response

PROCHEŞ *ET AL.* POINT OUT THAT CORRIDORS may increase the spread of exotic species. We agree that the function of corridors is blind to the geographic origin of species that use them.

The issue before conservation biologists and land managers, however, is not whether corridors are without costs, but whether they provide a net benefit in the maintenance of natural communities. In this context, it is important to keep in mind that the benefits of habitat corridors to native species have been clearly demonstrated, whereas their impact on the spread of exotic species is largely conjectural.

Rather than debating the potential drawbacks of corridors, scientists should focus attention on understanding how corridors function and which types of species are most likely to benefit from them. For example, because invasive species are excellent dispersers (by definition), corridors may not further increase their successful colonization of new habitat patches. On the other hand, many native species of conservation concern have limited dispersal abilities and therefore would be more likely to benefit from corridors.

Understanding corridors at a mechanistic level will better enable us to extrapolate their effects from well-studied species and small spatial scales to less-known species and landscape scales; our paper aimed toward this goal.

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

TECHNICAL COMMENT ABSTRACTS

COMMENT ON "A Brief History of Seed Size"

Peter J. Grubb, David A. Coomes, Daniel J. Metcalfe

Moles *et al.* (Reports, 28 Jan. 2005, p. 576) suggested that larger plants have larger seeds because larger offspring offset the lower survivorship to adulthood inherent in longer juvenile periods. However, that view is not consistent with the wedge-shaped relationship between log seed size and log plant height. Most importantly, the range of feasible seed sizes increases dramatically with whole-plant size.

Full text at www.sciencemag.org/cgi/content/full/310/5749/783a

RESPONSE TO COMMENT ON "A Brief History of Seed Size"

Angela T. Moles, David D. Ackerly, Campbell O. Webb, John C. Tweddle, John B. Dickie, Mark Westoby

Mechanical constraints might prevent small plants from making very large seeds. However, data for 2589 species reveal an absence of large plants that make very small seeds. This cannot be explained by mechanical constraint. Coordination of life history traits provides a more plausible explanation for the overall shape of the relationship between seed mass and plant size.

Full text at www.sciencemag.org/cgi/content/full/310/5749/783b

OPERA: PHYSICS

An Explosion in the Desert

Gregg Herken

The life of physicist Robert Oppenheimer has been the subject of numerous biographies, several novels, and a handful of plays. So it is perhaps not surprising that Oppenheimer should now inspire an opera, by composer John Adams and director-librettist Peter Sellars. The choice, both of artists and subject, is apt. In an earlier opera, *Nixon in China*, Adams took as his topic a moment where history took a sudden and unexpected turn. It would be hard to dispute that the advent of the atomic bomb was another such moment. Similarly, the story of Oppenheimer has always seemed peculiarly suited to drama. "Oppie" was a complex and conflicted figure who sought refuge from his many demons in Eastern religion, left-wing politics, and poetry.

Poetry is, appropriately, one of the main sources that Adams and Sellars draw upon. The lyrics to the opera's songs are actually lines from John Donne, Muriel Rukeyser (a mid-20th-century American poet who, it turns out, went to school with Oppenheimer), and the *Bhagavad Gita* interspersed with fragments of declassified documents dealing with the bomb. [It was at first a bit jarring to hear the chorus sing portions of the Smyth Report, *Atomic Energy for Military Purposes* (*1*), with cymbal and drum accompaniment, but, on reflection, it began to seem wholly appropriate.]

Because the opera concerns itself only with the few hours before the test of the bomb in the New Mexico desert, Adams's Oppenheimer (sung by Gerald Finley) seems destined to be a pared-down and oversimplified version of the man. Missing in this portrait are the radical leftist intellectual of the 1930s and the tortured paramour of Jean Tatlock, Oppie's lover before he met and married Katherine "Kitty" Puening (Kristine Jepson). Except for one brief mention of "certain scientists of doubtful discretion and uncertain loyalty," there is little hint of Oppenheimer's colorful and troubled past.

But the prospect of "Oppie Lite" is dispelled at the end of the first act, when the physicist sings Donne's *Holy Sonnet XIV* in a solo aria. (The poem's opening line, "Batter my heart, three-person'd God," is what prompted Oppenheimer to name the New Mexico test site Trinity, in a secret tribute to Tatlock, who had committed suicide a short time before.) The aria and the scene—which has Oppenheimer and the bomb silhouetted against the canvas that shrouded the device during its final assembly—are simultaneously haunting and stunning.

Also impressive is Adrianne Lobel's stage setting, which manages to convey changing perspective at the test site by means of a simple curtain backdrop and imaginative lighting. The main prop is, of course, the "gadget" itself, an eerily accurate rendition of the casing of the plutonium implosion device tested at Trinity. Beginning with the second act, the bomb becomes a kind of centerpiece on stage, at one memorable point hanging Damocles-like above the crib of Oppenheimer's sleeping infant daughter, Tony.

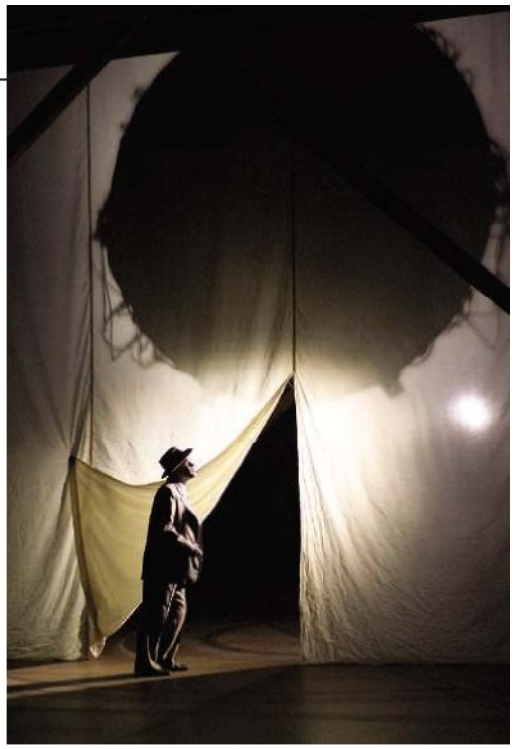
For all of its artistry, the opera does drag in places. In particular, Kitty's monologue, borrowed from Rukeyser's poems, is lush with imagery but so obscure in meaning as to be incomprehensible. (This is less of a problem with Oppenheimer, who was famous for his elliptical utterances. Aware that their telephone conversations were being monitored by government agents, Oppenheimer's lawyer used to blurt out in frustration to his client: "For God's sake, Robert, just say what you mean!")

Dr. Atomic
John Adams, composer. Libretto by Peter Sellars

San Francisco Opera, co-production with Lyric Opera of Chicago and De Nederlandse Opera, Amsterdam. San Francisco, 1 to 22 October 2005. www.doctor-atomic.com

There is also a scene in the second act when General Leslie Groves (Eric Owens), the military head of the bomb project, wanders on at length about his failed diets. While the Groves of Adams's opera is apparently meant to exude menace, the figure as portrayed comes across instead as slightly goofy. Not so Edward Teller, who is played by Richard Paul Fink with pitch-perfect fidelity as the ultimate no-nonsense pragmatist—even to the point of applying sunscreen just before the bomb explodes, to protect against its ultraviolet rays.

Predictably, the final scene, when the bomb goes off, is something of a letdown. While set designers have successfully replicated the sinking of the *Titanic* and the scaling of K2 for theater audiences, a convincing representation of a nuclear explosion is a dramaturgical challenge of a different order. Still, Adams and Sellars give it a rousing try. They elected to go with a kind of Zen approach rather than pyrotechnics. Discordant noises and electronic music finally resolve into the single, plaintive voice of a woman speaking Japanese, foreshadowing the event that we all know is coming. (But an English translation on the monitors that flank the stage would have been helpful. According to my multilingual seatmate, the woman is asking for help and pleading for water.)



In the hours before dawn. Oppenheimer and the bomb at the end of Act I.

CREDIT: TERRENCE MCCART MCCO...RTESY SAN FRANCISCO OPERA

Doubtless, scientists as well as historians will find things to quibble with in *Dr. Atomic*. The opera's opening lines—"Matter can be neither created nor destroyed but only altered in form"—drew the attention of the president of the American Physical Society, who includes in the printed program a correction along with an endorsement of the opera. More difficult to explain away is the opera's portrayal of the guilt the scientists supposedly felt for their role in creating the bomb. Here Adams chose Robert Wilson (Thomas Glenn), a young physicist and group leader at Los Alamos, to represent the voice of conscience. (Wilson was a shy and sensitive man, but the role might have been better occupied by Kenneth Bainbridge, the gruff physicist whom Oppenheimer had put in charge of Trinity. Bainbridge could have sung, sotto voce, what he actually told Oppenheimer in the minutes after the explosion: "Well, Oppie, now we're all sons of bitches.")

In the opera, Wilson urges a demonstration of the bomb as an alternative to dropping it on

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a Japanese city. But he is overruled, on practical grounds, by Oppenheimer. When the test goes off, the character playing Wilson lies prostrate on stage, rendered immobile not so much by the physical power of the bomb as by the moral implications of what he and his colleagues have wrought. It is true that Wilson was an advocate of a demonstration, and likewise true that he became physically ill upon hearing the casualty reports from Hiroshima. But, in an interview more than 20 years ago, the real Robert Wilson recalled with undisguised glee how he and his fellows had jumped into a jeep shortly after the bomb went off and raced toward the crater formed by the explosion, en route "making rude Italian gestures" to Enrico Fermi (whose lead-lined tank had broken down on its way to ground zero).

Like Oppenheimer, with his famous "high-noon strut" in the immediate aftermath of Trinity, for Wilson, the doubts only came later. At the moment, all qualms and reservations were submerged in the celebration of the weapon's technical perfection. That is the really scary part about the making of the atomic bomb, and it's not in the opera.

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10.1126/science.1121537

PLANETARY SCIENCE

Impressions of Our Solar System

Donald K. Yeomans

The field of planetary science is currently enjoying an intense period of paradigm readjustment and discovery. We no longer believe that the major planets Jupiter, Saturn, Uranus, and Neptune formed a few billion years ago in their current locations. Instead, after Jupiter formed through an agglomeration of primordial icy comets, its gravitational interaction with the remaining comets threw millions of them out of the solar system. In the process, Jupiter moved sunward from its original orbit to conserve angular momentum while Saturn, Uranus, and Neptune moved outward from their birthplaces. Neptune's early passage through the region where cometary planetesimals formed created a 100-million-year period of destabilization whereby these icy objects and asteroids bombarded Earth. One collision with a Mars-sized object created our Moon, and the intensity of the bombardment frustrated life on Earth's surface until between 4 billion and 3.5 billion years ago.

In terms of discoveries, the last decade has seen the detection of more previously unknown planets, satellites, asteroids, and comets than the previous several centuries combined. We now note about three small comets colliding with the Sun each week. Some comets have been observed to disintegrate completely for no apparent reason, whereas others have apparently lost their ability to outgas and now appear to be asteroids. For their part, about 5000 new asteroids are now reported each month, and a few move in comet-like orbits. A surprising number of the asteroids have been found to have their own little moons. At least one, asteroid 87 Sylvia (named after the mother of the mythical founders of Rome), has twin moonlets (named after the founders, Romulus and Remus).

The total number of natural satellites orbiting the major planets has grown to more than 150, with more than 50% of these discoveries occurring within the last 6 years. Since 1992, more than 800 previously unknown icy worlds have been discovered in the region beyond Neptune and at least one of them (temporarily designated 2003 UB313) is larger than Pluto. This new object also has its own moon. Pluto itself—until recently our ninth and outermost planet—may be in jeopardy of losing its planetary status or it may only lose its status as the outermost planet. A committee, formed by the International Astronomical Union to decide whether some of these discoveries should be termed new planets, has not yet been able to provide a consensus view on just what a planet is. A workable definition for a planet is particularly important because since 1995 more than 150 planet-like objects have been discovered orbiting stars outside our own solar system.

The Planets
by Dava Sobel
Viking, New York, 2005. 284 pp. $24.95, C$35. ISBN 0-670-03446-0. Fourth Estate, London. £15. ISBN 1-85702-850-3.

Into this current climate of planetary science rethinking and intense discovery, Dava Sobel introduces a personal and retrospective look at the nine traditional planets and a few of these new discoveries. In her rather short book, nearly devoid of illustrations, the best-selling author weaves mythology, astrology, history, and analogies into word pictures of the Sun's family of planets. For the most part, Sobel's impressionistic portraits are successful. Easily read, the book is stuffed with interesting comments, anecdotes, and notes. To mention a few examples: Galileo's 1610 discovery of four moons orbiting Jupiter marks the divergence of astronomy and astrology, because it suggested a heliocentric solar system whereas astrology demanded a geocentric viewpoint. While William Herschel scanned the heavens from his garden in Bath, England—observations that resulted in the discovery of Uranus and two of its satellites during the 1780s—he sought protection from the damp night air by rubbing his skin with an onion. James Clerk Maxwell is the only male to be honored in the names of surface features on Venus, an honor bestowed in the 1960s after the discovery of 5-mile-high peaks that were detected with Earth-based radar (observations that depended on Maxwell's pioneering work on electromagnetic radiation a century earlier).

Planetary system after Tycho Brahe, from Andreas Cellarius's *Harmonia Macrocosmica* (Amsterdam, 1661).

Now and then, Sobel's account becomes a bit strained, as in the chapter on Mars, where the story is told from the point of view of the ancient martian meteorite Allan Hills 84001. Some downright strange prose arises in the chapter devoted to the Moon, where we learn that a friend of the author swallowed some lunar dust given to her by an astronomer who was studying surface samples collected by the Apollo project. In a reverie, Sobel imagines, "As it entered her mouth, it ignited on contact with her saliva to shoot sparks that lodged in her every cell. Crystalline and alien, it illuminated her body's dark recesses like pixie powder, thrumming the senseless tune of a wind chime through her veins." One must wonder what that is all about.

Such minor quibbles aside, Sobel's relaxed stroll through the rapidly changing planetary landscape is well worth the read, and *The Planets* should provide newcomers an effortless introduction to its eponymous topic.

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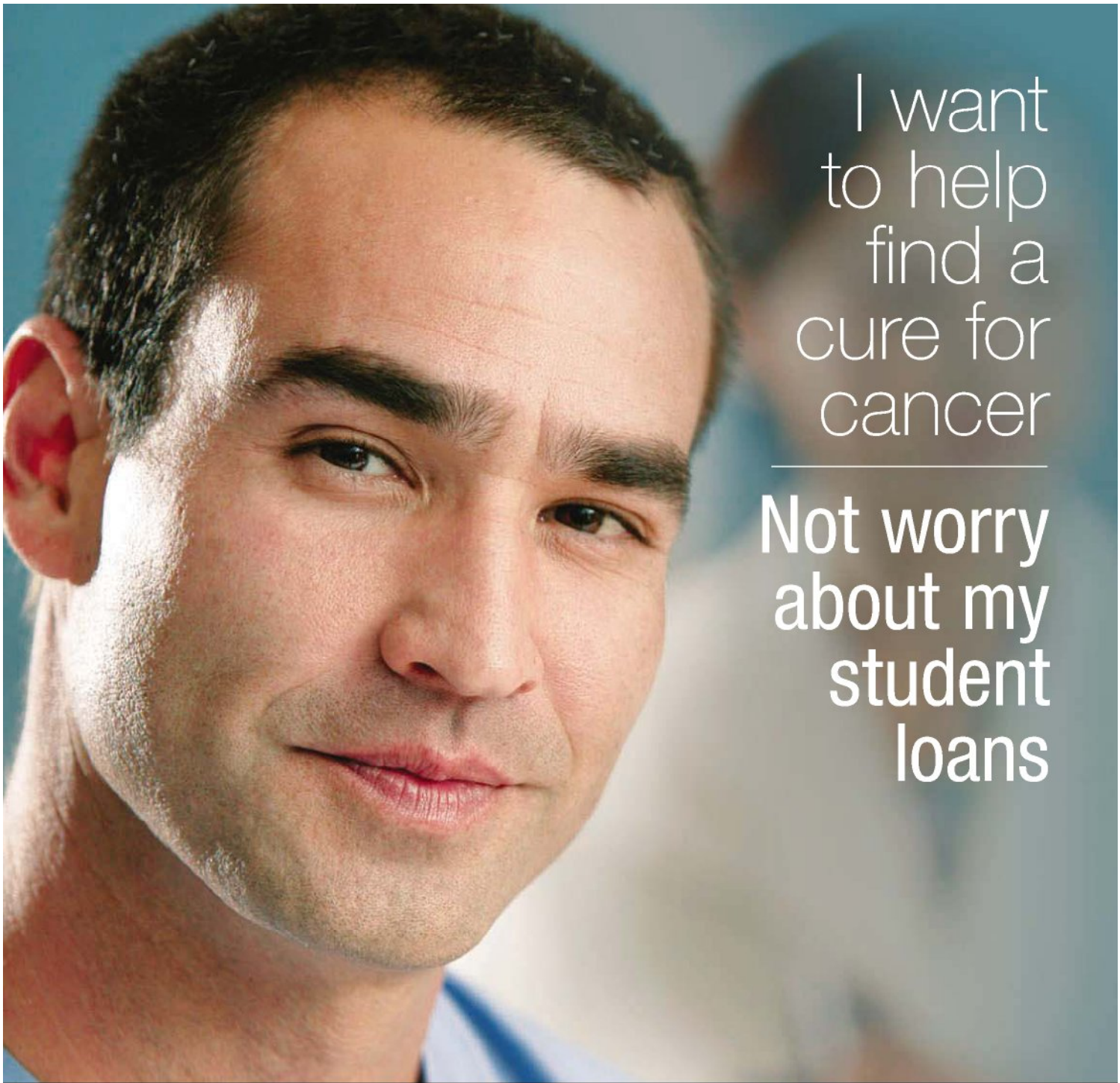

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Teaching Evolution in Mexico: Preaching to the Choir

Antonio Lazcano

In some of his writings, Charles Darwin expressed his interest in visiting Mexico. Although he never fulfilled that wish, Mexicans have reciprocated his interest with a long-standing commitment to his ideas. Based on the common misapprehension that Mexico's strong Catholic background has led to a rejection of evolution, many people in the United States remain convinced that teaching and research on the origins of life must be severely limited in my country. Deriving in part from Spain's Black Legend—in which the stunning intolerance exercised by the Inquisition became unfairly viewed in subsequent centuries as iconic of the country and its colonial exploits as a whole—this self-assuring American prejudice has led many uninformed observers to believe that today's Mexicans are the intellectually suffocated children of the Counter-Reformation, still ruled by a taciturn Papist church that rejects the notion of Darwinian evolution and other major scientific advances while clinging to its theological obsessions.

I am always amused when I am asked by my American colleagues about the problems and pressures they imagine I face in Mexico because of my interest in life's beginnings. However, pressure to include creationism in public pedagogical and research settings has been primarily a phenomenon in the United States. Only twice during my 30 years of teaching about evolutionary biology and research into the origins of life, have I encountered religious-based opposition to my work. In both cases, it came from evangelical zealots from the United States preaching in Mexico. One of the little recognized U.S. imports into Mexico is a small flow of creationists, who, through religion, are trying to impose their fundamentalist beliefs and hinder the teaching of Darwinian evolution in all levels of schooling.

It is true that the arrival of Darwinism was an unsettling event for a number of Latin American Catholics, and led to criticism from various sectors of the Church. However, historians have recorded no major controversies developing in Mexican society after the publication in 1859 of *The Origin of Species*. Such quietude stemmed in part from the fact that Rome does not advocate the literal reading of the Bible the way Protestant evangelists do. With time, the clash between the Old Testament and Darwin's ideas faded into a more or less peaceful coexistence between the theories and discoveries of evolutionary biology, on the one side, and the teachings of the Church, on the other. Although it might not be generally or frequently acknowledged, there has been an age-old tradition of compatibility between science and the Catholic Church. The Galileo affair stands out as an anomalous moment of extreme intolerance.

Of course, neither the Church nor its members are monolithic entities. As in other places with a strong Catholic background, such as France, Italy, Spain, and most Latin American countries, Mexican society as a whole is not only predominantly secular, but it also takes for granted the existence of strong laical institutions. This is a subtle but important distinction that explains why Mexico and many largely Catholic countries succeed at maintaining an extended form of secularism while also supporting religious freedom. This works so long as citizens in these countries express this freedom within the realm of their personal beliefs and not within a context of public policy-making. It helps here that in Latin America most Catholics tend to read the Old Testament not as the literal truth, but as a depiction of the ways in which divine creation may have taken place. It is thus possible to be a Catholic Bible-reader, or more generally a believer in the supernatural origin of life, without being a card-carrying creationist who has to reject Darwinian evolution in order to maintain logical consistency within a framework of fundamentalist Christian premises.

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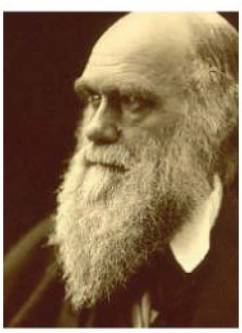
Antonio Lazcano
Mexico

Antonio Lazcano, a biology professor at the Universidad Nacional Autónoma de México (UNAM) in Mexico City, has studied the origin and early evolution of life for more than 30 years. He was trained both as an undergraduate and graduate student at UNAM, where he focused on the study of prebiotic evolution and the emergence of life. An academic deeply committed to public education, he has devoted considerable efforts to scientific journalism and teaching. He is the author of several books published in Spanish, including *The Origin of Life*, first printed in 1984 and which has become a bestseller with more than 600,000 copies sold. He is an avid promoter of evolutionary biology and the study of the origins of life in Latin America, and has been professor-in-residence or visiting scientist in France, Spain, Cuba, Switzerland, Russia, and the United States. In addition, he has served on many international advisory and review boards, including ones for NASA and other international organizations. He has just been reelected president of the International Society for the Study of the Origin of Life, the first Latin American scientist to occupy this position.

All essays and interactive features appearing in this series can be found online at www.sciencemag.org/sciext/globalvoices/

A Love Affair with Darwin

The study of the origin of life and other issues of evolutionary biology run deep in Mexican culture. This shows up in many ways, including Diego Rivera's cheerful mural paintings of Charles Darwin in public buildings and the popularity of Aleksandr Oparin's ideas about life emerging from a primordial soup. More than 70 editions of *The Origins of Life*, one of Oparin's earliest books, have been published here and read by generation after generation of high-school students since it was first translated in 1937. Perhaps even more important is the nationwide exposure for many decades of Mexico's schoolchildren to evolutionary ideas included in the textbooks published by the Mexican Secretary of Public Education, which are provided free to all students. The lessons based on these materials are a preamble to in-depth teaching of evolution in secondary (middle school) and high schools.



What a guy. In Mexico, Darwin doesn't get a lot of grief.

In the early part of the 20th century, the Mexican naturalist Alfonso L. Herrera (1865–1942) became one of the most active early popularizers of evolutionary ideas. With relentless energy, he lectured, wrote, and established public museums devoted to the promotion of Darwinism. He also contributed to the science of evolutionary biology by developing a theory on the autotrophic origins of life, according to which the first cells had been endowed since their emergence with the plantlike ability to synthesize their own components from carbon dioxide. Although none of Herrera's associates built upon his theory, he had a lasting influence in Mexican biology. Many years after he died, his contributions are still acknowledged, a fact that indirectly helped my own early professional development.

Some 30 years ago, I became intensely interested in the prebiotic significance of extraterrestrial organic compounds, and decided to teach a course on the origins of life at the Universidad Nacional Autónoma de México (UNAM). In large part because of the intellectual foundation Herrera had laid down many decades ago, and the sympathy that Darwin's ideas inspire in Mexico, my proposal to teach the course—in spite of my youth and lack of experience—was greeted with considerable enthusiasm by my colleagues, the university administration, and the students. To this day, new generations of students continue to flock to this and other courses on evolutionary biology.

In yet another sign that Mexico's educators and students embrace Darwinism, my associates and I are often invited to speak in public and private schools, including those run by Catholic nuns and priests, to talk about the origin and evolution of life. The list of venues includes a conference at the oldest Mexican Catholic seminary. Many of the students and professors at the seminary may have seen evolution as the unfolding of a divine plan, but they also saw no doctrinal conflict between their own personal faith and Darwin's scientific ideas. They even found hilarious the idea of teaching creationism based on biblical literalism.

As shown by the opinion article published on 7 July 2005 in the *New York Times* by Christoph Cardinal Schönborn, not all members of the Catholic hierarchy feel comfortable with the premises and results of evolutionary theory. It is equally true that some Church thinkers and theologians have tried to criticize the philosophical tenets of evolutionary theory, but most tend to accept the results of experimental research and the general evolutionary framework, while maintaining a spiritualist stand. This attitude, which has been prevalent among Vatican theologians especially since the times of Pope Pius XII in the middle of the last century, owes much to the intellectual sophistication of orders like the Jesuits and the Dominicans.

In his famous 1996 address to the Pontifical Academy of Sciences, the late Pope John Paul II acknowledged that the theory of evolution is not a mere hypothesis, while also reiterating the supernatural origin of the human soul. By shifting emphasis from creation per se, to the origin of the soul, Pope John Paul II found a relatively safe common ground to stand on, since scientists are entirely unable to prove (or have no interest in proving) the existence or nonexistence of the soul. In spite of such subtleties, most Mexican Catholics clearly do not view the premises and developments of evolutionary theory as a battleground or as major theological risk. Stealing the spotlight for the moment for Mexican Catholics and other Christians are ethical controversies associated with new and emerging biotechnologies, especially those based on stem cells, fertility research, and genetic manipulation

Science Be Damned

It is hard for Mexicans to understand the hold that religion has in America, and many of us are baffled by the lax attitude of policymakers in the United States to the religious right, who manage to influence and sometimes undermine the public educational system. Thomas Jefferson's famous phrase about "the wall of separation" between the Church and State may be a guiding principle of American politics, but the huge cultural space that evangelical Protestantism and other politically active religious movements have gained in the United States demonstrates how tenuous are the boundaries between the secular and the religious.

As summarized by Noah Feldman in his book *Divided by God*, the belief that the Old and New Testaments were literally and verbally inspired is deeply rooted in American mainstream culture, and remains a pervasive influence in many aspects of everyday life, including elementary and higher education. In contrast, Mexico still maintains some anticlerical attitudes, and public education bears the secular trademark of the Enlightenment, whose introduction into the country was facilitated by some prominent priests and Jesuits.

Feldman's thesis itself has deep roots. "For more than a thousand years," wrote Thomas H. Huxley in 1843 in the preface to his book *Science and Hebrew Tradition*, "the great majority of the most highly civilized and instructed nations in the world…have held it to be an indisputable truth that, whoever may be the ostensible writers of the Jewish, Christian, and Mahometan [Islamic] scriptures, God Himself is their real author; and, since their conception of the attributes of the Deity excludes the possibility of error and—at least in relation to this particular matter—of willful deception, they have drawn the logical conclusion that the denier of the accuracy of any statement, the questioner of the binding force of any command, to be found in these documents is not merely a fool, but a blasphemer. From the point of view of reason he grossly blunders; from that of religion he grievously sins."

Although many American churches appear to reject the fundamentalist campaign against Darwinism, some of the most aggressive versions of creationism—including the latest one dubbed "intelligent design" by its champions—have been growing rapidly in the fertile soil provided by some of the evangelical churches that sprung up in the 19th and early 20th centuries. The United States is unique among Western countries for its religiosity. Polls consistently show that only a small percentage of Americans hold a secular view of the world, compared with an overwhelming 40% of the population that believes in strict biblical creationism.

This explains in part why following the 1987 United States Supreme Court ruling that opposed the teaching of so-called creation

science in the classroom, a new, recycled, highly pragmatic creationism has evolved (if you pardon the pun). It is a movement that has eliminated open references to Christianity; built networks of lecturers and researchers that propagate the creationist theology; introduced new players like the intelligent design movement; found major sources of funding from foundations run by politically active Christian conservatives; and adapted its fundamentalist literalism not only to the rhythm of pop music but also to the Web.

Their accomplishments can be measured not only by their emerging success in undermining the separation of Church and State in the context of science education in public schools in some states like Kansas, Ohio, and Pennsylvania, but also in the statements by major political figures, including President Bush, that attempt, if not to appease the religious right, at least to assure the public of their unwillingness to take a firm stand in support of evolutionary theory.

Darwin's place. At this elementary school, named Evolución, in the small Mexican city of Pachuca, children celebrate Darwin's birthday (12 February) with a ceremony and display of murals on his life and theory.

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Dangerous Exports

Since we can never know in full detail how the origin of life took place, it is not surprising that it is becoming a target for intelligent design creationists. The geological and chemical evidence required to understand life's beginnings remains insufficient and difficult to understand. For creationists, that evidentiary gap provides an opportunity to erect a framework of controversy and endless discussion around the study of prebiotic evolution and the origin of life, which they assume are best explained by an intelligent cause rather than by an undirected process like natural selection.

One of the little recognized U.S. imports into Mexico is a small flow of creationists, who, through religion, are trying to impose their fundamentalist beliefs and hinder the teaching of Darwinian evolution in all levels of schooling.

It is true that there is a huge gap in the current descriptions of the evolutionary transition between the prebiotic synthesis of biochemical compounds and the last common ancestor of all extant living beings. Even the unanticipated discovery in 1982—by the research teams directed by Thomas Cech and Sidney Altman—of catalytic RNA molecules (ribozymes), which can be loosely described as nucleic acids that simultaneously have characteristics of DNA and enzymes, has not closed this gap. Instead, that and related discoveries have led to a more precise definition of what should be understood as the origin of life. The origin of protein synthesis is still not understood, but the surprising conservation of widely distributed polypeptide sequences related to RNA metabolism has led my group and others to suggest that these sequences provide insights into an RNA/protein world that may have resulted from the interaction of ribozymes with amino acids, and that very likely preceded our familiar DNA/RNA/protein world. Our understanding of the origin and early stages of biological evolution still has major unsolved problems, but they are recognized by the scientific community as intellectual challenges, and not as requiring metaphysical explanations, as proponents of creationism would have it.

Scientists from other countries could take a certain solace in the fact that the creationist movement appears to be largely confined to the United States. I find it extremely encouraging that Mexican students, for the most part, are not driven by gaps in the scientific view of life to search for religious explanations or to vitiate evolutionary theory by advocating intelligent design. Our teachers and pupils alike generally view the framework of intelligent design as a thinly disguised attempt to introduce religious preconceptions into the classroom. Even so, it would be unwise to simply sit back and watch with incredulity as our American colleagues struggle against intelligent design creationists and other fundamentalisms. There are, in fact, manifold indications that the creationism movement has been flexing its muscles and looking to proselytize far and wide. Its potential threat to science education in Mexico and other Latin American countries should not be underestimated.

In the United States, Hispanics account for 14% of the population, but the demography of American science does not reflect this figure. The success of the American educational system in attracting Latinos (many of whom live in the Bible belt) into science careers has been limited, but the evangelical movement has not lost time in recruiting them. Its progress in the United States has been extended by many fundamentalist Mormons and Pentecostalist missionaries who travel abroad to search for adherents in other countries. Their followers now include growing numbers of legal and illegal Mexican migrants, driven by the American dream, who go back and forth across the border. Steeped in the parochial thinking of biblical literalism, the open commitment by these missionaries to impose nonsecular views in education is an indication of a looming confrontation in both countries. Tall fences make good neighbors, but stronger new forms of cooperation between the academic communities on both sides of the Mexican-American frontier could do better.

Creationism is a danger to science education that should be addressed by a constructive dialogue and collective actions led by imaginative researchers and educators on both sides of the border. Our answer to the fundamentalist challenge could include better academic exchange programs, common strategies designed to promote the teaching of evolutionary biology, and joint outreach activities for both Mexican and U.S. Latino students, who share important cultural backgrounds. The potential benefits of such common strategies could be manifold, including a proper honoring of the freedom of all to follow (or not) religious beliefs, while rendering to Caesar the things that are Caesar's, to God the things that are God's,...and to Darwin those that are Darwin's.

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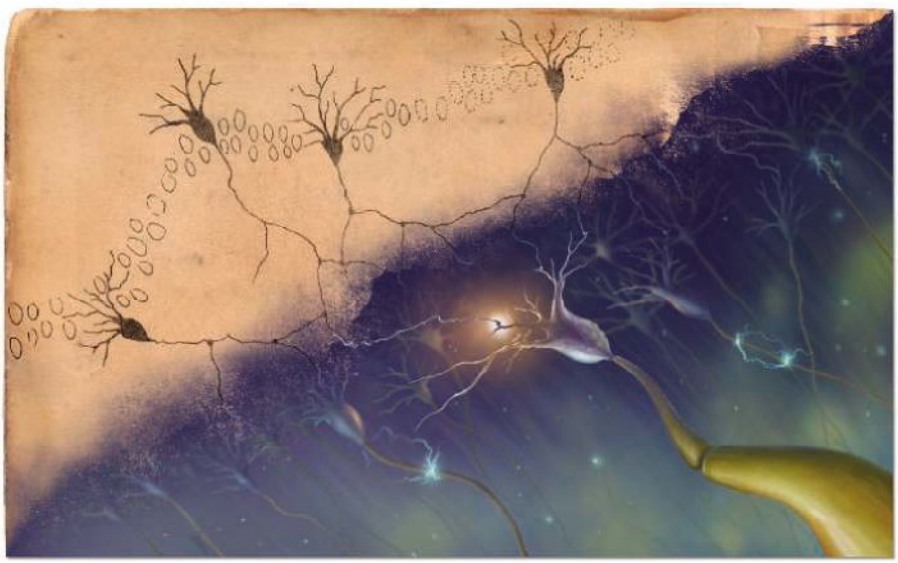
NEUROSCIENCE

The Neuron Doctrine, Redux

Theodore H. Bullock, Michael V. L. Bennett, Daniel Johnston, Robert Josephson, Eve Marder, R. Douglas Fields

After a century, neuroscientists are rethinking the Neuron Doctrine, the fundamental principle of neuroscience. This proposition, developed primarily by the great Spanish anatomist and Nobel laureate Santiago Ramón y Cajal, holds that a neuron is an anatomically and functionally distinct cellular unit that arises through differentiation of a precursor neuroblast cell. In principle, part of this tenet has held up, but technology and research have extended our knowledge far beyond this simple description. What has evolved is a modern view of the neuron that allows a more broad and intricate perspective of how information is processed in the nervous system. One hundred years since its inception, an examination of the Doctrine indicates that it no longer encompasses important aspects of neuron function. If we are to understand complex, higher level neuronal processes, such as brain function, we need to explore beyond the limits of the Neuron Doctrine.

In the early 20th century, the nervous system was thought to function as a web of interconnected nerve fibers. The cytoplasm and nervous impulses were thought to flow freely in any direction through the network of fibers. But it was Cajal who envisioned the neuron as an individual functional unit, polarized such that signals are received through its rootlike dendrites and transmitted through its long axonal process. He posited that although an axon terminates adjacent to a dendrite of the next neuron (see the figure), the cleft between them would act as a synaptic switch regulating information flow through neural circuits. The synaptic cleft went unseen until a half-century later, when in 1954 the electron microscope provided convincing evidence that essentially refuted the earlier "reticular" view of a nerve fiber web (*1*).



Information processing, past and present. The Neuron Doctrine transformed the 19th-century view of the nervous system which saw the brain as a network of interconnected nerve fibers (**upper left**). A century later, the modern view (**lower right**) holds the neuron as a discrete cell that processes information in more ways than original envisaged: Intercellular communication by gap junctions, slow electrical potentials, action potentials initiated in dendrites, neuromodulatory effects, extrasynaptic release of neurotransmitters, and information flow between neurons and glia all contribute to information processing.

At the same time, physiological studies established that conduction of electrical activity along the neuronal axon involved brief, all-or-nothing, propagated changes in membrane potential called action potentials. It was thus often assumed that neuronal activity was correspondingly all-or-nothing and that action potentials spread over all parts of a neuron. The neuron was regarded as a single functional unit: It either was active and "firing" or was not.

This dogma began to erode with the advent of microelectrodes that could be inserted into neurons to record electrical signals. In 1959, it was realized that much of the information processing by neurons involves electrical events that are graded in amplitude and decay over distance, rather than all-or-nothing electrical spikes that propagate regeneratively (*2*). It was also determined that evoked electrical responses often occur on a background of spontaneous changes in membrane potential (i.e., produced without input from other neurons) and that some parts of the neuron are incapable of producing all-or-nothing action potentials (*3*). Today, it is apparent that information processing in the nervous system must operate beyond the limits of the Neuron Doctrine as it was conceived. This has evolved from detailed information gained from techniques developed in the past 50 years—notably single-channel recording, live cell imaging, and molecular biology.

Although Cajal wisely considered that "neuronal discontinuity… could sustain some exceptions" to the Doctrine's definition (*4*), he could not have foreseen the presence and role of neuronal gap junctions as one of these exceptions. These assemblages of protein pores form small aqueous channels of limited selectivity that connect neurons, providing cytoplasmic continuity (*5*). We now know that gap junctions are widespread in the mammalian nervous system (*5*) and function to synchronize neuronal firing. They constitute electrical synapses that couple groups of cells into functional syncytia—in this

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sense, the reticular concept, reinvoked. Electrical transmission through gap junctions was initially considered primitive and likely incapable of the subtleties of chemical transmission through axon-dendrite synapses (early studies showed that synapses with gap junctions between the axon of one neuron and the cell soma of another neuron also have regions resembling the active zones of chemical synapses, although there is no chemically mediated signal transmission and response). Although gap junctions can behave as simple electrical resistances between connected cells, an electrical impulse in one cell by no means inevitably propagates to the other cells with which it shares gap junctions. In fact, a channel within a gap junction is not necessarily open, and an entire gap junction may not transmit electrical current until it is appropriately modified in response to transmission from chemical synapses of the same, "presynaptic" neuron. This modulation of channels provides electrical synapses at gap junctions with the plasticity long considered an exclusive province of chemical synapses at axon-dendrite junctions (*6*). Furthermore, gap junctions have been described between neurons and non-neuronal cells such as astrocytes (*7*), a somewhat controversial finding not conceived in the original Neuron Doctrine.

Fifty years ago, neuroscientists also did not realize that a plethora of neuromodulatory substances, such as amines and neuropeptides, can reconfigure neuronal circuits into different patterns of functional connection, capable of a variety of activity patterns (*8*). Almost all neurons and synapses are subject to such neuromodulation, which acts to remodel neuron behavior and circuitry within minutes and hours rather than on the millisecond time scale typical of electrical impulse transmission. Many behaviors, including learning and memory, sexual cycles, mood, and sleep, occur over much slower time scales relative to processes such as reflexes or sensory and motor function. In addition, neuromodulatory substances can act at multiple sites on the neuron, including the axon. For example, some crab (*9*) and lobster (*10*) axons have receptors to amines such as dopamine, serotonin, and octopamine. When these amines are applied to the axons, these areas can spontaneously initiate action potentials in a nonclassical mode of integration.

Research during the past 10 years has shown that in many neurons, action potentials can travel backward from the axon and soma regions into the dendrites (*11*). Moreover, under certain conditions action potentials can be initiated in dendrites, remaining local or sometimes propagating into the soma to initiate single or multiple spikes of activity in the axon (*12*). The functional complexity of dendrites and the roles they play in synaptic integration and plasticity are well beyond what could have been deduced from Cajal's anatomy or from later somatic recordings (*2*). Dendrites contain a mosaic of voltage-gated ion channels (*13*). The types, densities, and properties of these channels are very diverse among classes of neurons (and even within a single class), and these channels regulate, on wide-ranging time scales, how a neuron responds to the thousands of incoming synaptic events that impinge on its dendrites. Important questions for the future will be how the spatial distributions of individual ion channels in dendrites are established, how this localization changes in response to incoming synaptic inputs and output firing patterns (*14*), and how the channels dynamically regulate excitability during different behavioral states.

Cajal was also careful to distinguish neurons from the many other cells in nervous tissue. The function, origin, and diversity of non-neuronal cells eluded Cajal, because a staining method, which revealed neuronal structure with brilliant clarity, left major classes of non-neuronal cells invisible (including microglia and oligodendrocytes). It is ironic that today we understand that the fundamental tenet of the Neuron Doctrine—polarized communication between neurons by action potentials—is heavily influenced by non-neuronal cells. These are the constituents of the nervous system that form the myelin sheath around axons and organize ion channels into periodic clusters along the axon, features that facilitate action potential propagation (*15*).

Myelinating glia do not fire action potentials, but they can detect impulses in axons through membrane receptors that bind signaling molecules. These include ATP (*16*) and adenosine (*17*) that are released along the axon and also potassium that is released during intense neural activity. This axon-glial communication violates the Neuron Doctrine in two ways. Information is communicated between cells at sites far removed from chemical synapses, and it propagates in a transduced form through cells that are not neurons (*18*). In response to neural firing, glia communicate with other glia by chemical signaling and gap junctions rather than by electrical impulses (*18*). Unexpectedly, chemical synapses have recently been detected between neurons and a class of glia (oligodendrocyte precursor cells) (*19*), undermining a defining feature of neurons. However, the functional importance of this neuron-glia interaction is unknown. We now know that during vertebrate embryonic development, glia can give birth to neurons (*20*), challenging Cajal's conclusion that neurons develop only from neuroblasts.

Astrocytes are now known to communicate among themselves by means of glial transmitters and neuromodulators as well as by gap junctions (*18*). Moreover, astrocytes can detect neurotransmitters that are released from neuronal chemical synapses (*21*). These transmitters are delivered via synaptic vesicles into the synaptic cleft and diffuse to perisynaptic astrocytes. Additionally, neurotransmitters can be released outside the synapse and detected by perisynaptic glia (*22*, *23*). In response, astrocytes can regulate communication between neurons by modifying synaptic transmission through the release of neurotransmitters and neuromodulators (*18*). Thus, there may be a parallel system of information processing that interacts with neuronal communication but propagates over much slower time scales through a functionally reticular network of non-neuronal cells. This functional reticulum results from gap junction coupling and the omnidirectional communication that is mediated by chemical messengers released from astrocytes over much slower time scales. Such may be the case in the human brain.

Obviously, although neurons are indeed anatomically discrete units, they are not the single functional units in the sense envisioned by early proponents of the Neuron Doctrine. And the simplistic and static connectivity patterns described by Cajal and other cellular neuroanatomists must be revised in light of new information. The differences in specific membrane and cellular properties among cell bodies, axons, and dendrites, and even between different areas along dendrites, are far more extensive and sophisticated than would have been imagined nearly 50 years ago. Absolutely unforeseen a century ago is the active participation of non-neuronal constituents of the nervous system. A Neuron Doctrine reexamined hence provides a renewed perspective to ask many intriguing questions, particularly those about the human brain. For example, what features of the human brain account for our level of behavioral complexity? It is doubtful that the answer emerges from knowing the sheer number of cells, or the properties of synapses, or the identity of neurotransmitters and modulators. Such features are shared by many animals, especially vertebrates. There are, however, fundamental differences in electroencephalograms across the evolutionary spectrum—that is, in the electric field potentials arising from assemblies of functioning neurons. This suggests that the complexity of the human brain and likely other regions of the nervous system derive from some organizational features that make use of the permutations of scores of

integrative variables and thousands or millions of connectivity variables (*24*) and perhaps integrative emergents yet to be discovered. The answers extend well beyond explanation by the neuron acting as a single functional unit.

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STRUCTURAL BIOLOGY

A Ribosomal Coup: *E. coli* at Last!

Peter B. Moore

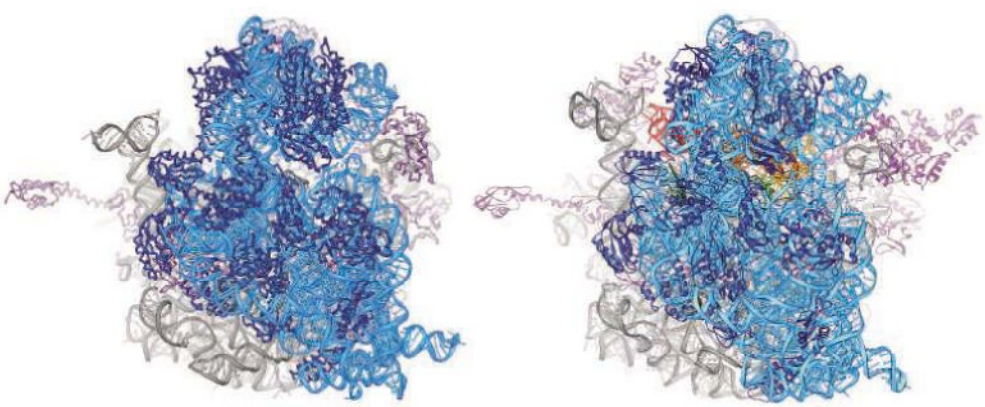
On page 827 in this week's issue, Schuwirth *et al.* (*1*) report an atomic resolution (3.5 Å) crystal structure for the 70*S* ribosome from the bacterium *Escherichia coli* (see the figure). More accurately, they report the atomic resolution for two such structures, because there are two, nonequivalent copies of the 70*S* ribosome per asymmetric unit in the crystals they have analyzed. The ribosome is the ribonucleoprotein enzyme that catalyzes messenger RNA–directed protein synthesis in all organisms, and the 70*S* ribosome, which is a 1:1 complex of a large and a small ribosomal subunit, is the particle that synthesizes proteins in prokaryotes. Because this enzyme plays a central role in gene expression, its structure has long been sought by molecular biologists.

The structures reported by Schuwirth *et al.* are by no means the first ribosomal crystal structures to appear. We already have a 2.4 Å resolution crystal structure for the large ribosomal subunit from *Haloarcula marismortui* (*2*), and a 3.1 Å resolution structure for the large ribosomal subunit from *Deinococcus radiodurans* (*3*). Two versions of the structure of the small ribosomal subunit from *Thermus thermophilus* have appeared, one at a resolution of 3.0 Å (*4*), and the other at a slightly lower resolution (*5, 6*). In addition, there is a structure for the 70*S* ribosome from *T. thermophilus* determined at 5.5 Å (*7*). Our sense of *déjà vu* is heightened by the impression that these new structures look very much like those that have appeared before (see the figure). Thus, we might wonder why these new structures should be considered noteworthy (which they are).

There are three reasons why these structures deserve attention. First, the structures that Schuwirth and colleagues have solved are that of the ribosome from *E. coli*. Since 1960, the *E. coli* ribosome has been the ribosome of choice for biochemists and molecular biologists; for no other ribosome is the information more complete. Observations made with the *E. coli* ribosome have been extensively used to interpret all the ribosome structures published previously, all of which came from other organisms. The argument has been that the extensive sequence homology that exists between ribosomes from different species justifies such cross-species comparisons. However, at some level, observations made on ribosomes from a mesophilic eubacterium like *E. coli* cannot be valid for ribosomes obtained from an extreme archaeal halophile like *H. marismortui*, or from an extreme eubacterial thermophile like *T. thermophilus*. These concerns can now be directly addressed.

Second, Schuwirth *et al.* are not the first investigators to attempt the crystallization of ribosomes from *E. coli*. For decades, laboratories all over the world have tried to obtain such crystals because of the obvious importance of the structures that might emerge from them. Schuwirth *et al.* are the first to obtain ribosomal crystals from this species that were worth analyzing, and that in itself is a coup. It should also be noted that the asymmetric unit of the crystals they have solved is gigantic; it contains roughly 5 megadaltons of macromolecular material. Determining structures this large is not trivial, even when much is known about them already, as was the case here.

Third, there is the matter of resolution. The resolution of the best 70*S* structure pub-



Structures of the 70S ribosome from two prokaryotes. (Left) *E. coli.* ribosome at 3.5 Å resolution [from (*1*)]. **(Right)** *T. thermophilus* ribosome at 5.5 Å resolution [from (*7, 9*)]. Both are oriented such that the small subunit [ribosomal RNA (light blue) and protein (dark blue)] is in the front.

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lished previously is 5.5 Å. In electron density maps in that resolution range, nucleic acid helices look like curved ribbons whose constituent nucleotides are often difficult to delineate, and protein density is hard to interpret at all. Nevertheless, a great deal was learned from those electron density maps because relevant structures that had been solved at higher resolution before could be fitted into them. The problem with the 70*S* model that emerged is that wherever its structure deviated from that of the structures being fit into its electron density maps, it was difficult to be sure what was going on. In 3.5 Å resolution electron density maps, such as those that led to the 70*S E. coli* structure reported by Schuwirth *et al.*, these ambiguities disappear because individual nucleotides are clearly visualized, and protein electron density is independently interpretable.

What has been learned? The structures presented by Schuwirth *et al.* are not the last word about the information contained in the particular crystals examined. Ribosomal proteins are not fully modeled at this point, and the structures are not fully refined. In addition, the crystals analyzed by Schuwirth *et al.* lack transfer RNAs or any of the other proteins, nucleic acids, or small molecules that interact with the ribosome during protein synthesis. Nevertheless, several themes clearly emerge. The structures of the bridges that hold the two subunits together are clear, which is important because the bridges are critical functionally: The two subunits of the ribosome not only communicate during protein synthesis, they also engage in coordinated, relative motions (*8*). In addition, the two 70*S* structures reported by Schuwirth *et al.* differ in the orientation of the head domains of their small subunits, and in neither is the head domain position the same as it is in the *T. thermophilus* 70*S* ribosome structure now available (*7*). Movements of the small subunit's head domain like the ones reported by Schuwirth *et al.* occur during protein synthesis [e.g., (*8*)]. It is now possible to understand how these motions occur at the molecular level, and to propose models for how they might be coupled to the events of protein synthesis. It remains to be seen what the small differences in conformation between the large ribosomal subunit of these *E. coli* ribosomes and the large ribosomal subunit structures of other organisms actually mean. Thus, the ribosome structures obtained by Schuwirth *et al.* really do advance our understanding of protein synthesis. Now that high-quality crystals are available for the *E. coli* 70*S* ribosome, the rate at which new information is obtained should increase.

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ATMOSPHERIC SCIENCE

Water Vapor Feedback in Climate Models

Robert D. Cess

General circulation models (GCMs) are the most detailed computer simulations available for projecting climate change caused by increasing greenhouse gases, as well as other anthropogenic changes. These numerical models contain numerous parameterizations of physical processes occurring within the climate system (that is, small-scale processes have to be described within the models). As a result, there is a need to devise ways of testing these parameterizations and processes within GCMs. On page 841 of this issue, Soden *et al.* (*1*) report an important reality check on one such process: the role of atmospheric water vapor in climate change.

It has long been known (*2*) that cloud-climate interactions constitute a major uncertainty in attempting to project future climate change with a GCM. As an illustrative example, if global cloud cover were to decrease because of climate warming, then this decrease reduces the infrared greenhouse effect due to clouds. Thus, the climate system is able to emit infrared radiation more efficiently, moderating the warming and so acting as a negative feedback mechanism. But there is a related positive feedback in this example that would increase the warming: The solar radiation absorbed by the climate system increases because the diminished cloud cover causes a reduction of reflected solar radiation by the atmosphere.

The situation is actually far more complicated than in this simple example, because changes in cloud cover will undoubtedly depend on cloud type and geographical location. Moreover, there would likely be associated changes in cloud altitude and cloud optical depth. One test of cloud-climate interactions within a GCM is to determine, relative to satellite observations, how well a GCM represents the radiative impact of clouds on the model's climate during the 5 years encompassing 1985 to 1989, and the top panel of the figure demonstrates that many models do rather poorly in this respect. And with regard to those models that do agree well with Earth Radiation Budget Satellite observations, it must be emphasized that this test is a necessary, but not sufficient, test of a model.

Another feedback mechanism is water vapor feedback. Water vapor is the atmosphere's dominant greenhouse gas, and a change in its concentration associated with a change in climate would alter the greenhouse effect of the atmosphere, thus producing a feedback mechanism. In 1967 it was proposed (*3*) that the atmosphere might conserve its relative humidity, and if so, this would lead to a positive feedback because a warmer atmosphere would contain more water vapor, thus amplifying the warming. And indeed, GCMs do tend to conserve global mean atmospheric relative humidity, as is shown for one such model in the bottom panel of the figure. But for more than a decade there has been considerable debate on this issue, with suggestions that water vapor feedback might actually be a negative feedback mechanism.

Soden *et al.* (*1*) present a very clever way of testing one aspect of water vapor feedback. As they point out, observed moistening trends in the lower troposphere have been linked to corresponding changes in surface temperature. But attempts to observe a moistening trend in the upper troposphere have proven to be unsuccessful, and this is the issue that Soden *et al.* address. They accomplish this by using clear-sky satellite radiance measurements from the High Resolution Infrared Radiometer Sounder channel centered at 6.7 μm (channel 12), which measures a portion of the 6.3-μm water vapor absorption band and therefore is sensitive to water vapor in the upper troposphere. They then compare the channel 12 observations of global mean blackbody temperature, for the

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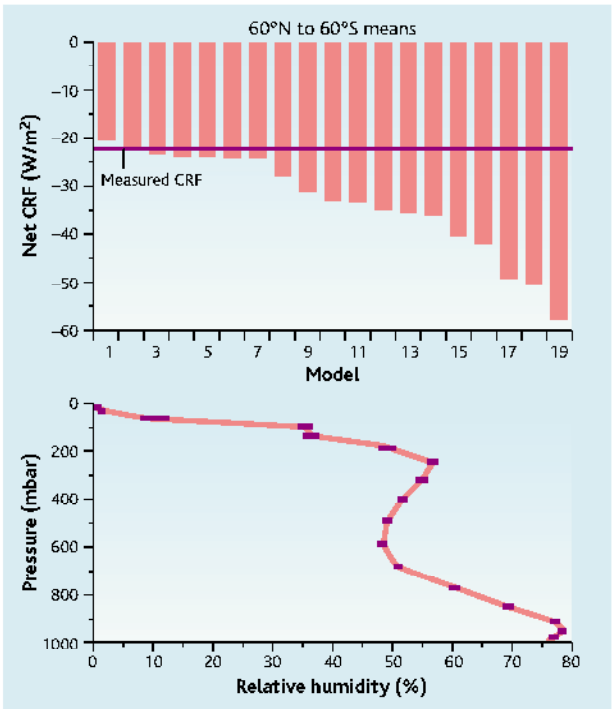

Cloudy predictions. (**Top**) Actual effect of clouds on climate (measured CRF) compared to the effect predicted by 19 global climate models. Some of the models significantly overestimate cloud-induced cooling. Clouds can potentially cool climate (by reflecting solar radiation) and simultaneously heat the system (by increasing the atmospheric greenhouse effect). The net effect illustrated in the figure is cooling, as indicated by the negative values of CRF. Actual net CRF (cloud-radiative forcing), measured by the Earth Radiation Budget Satellite (*4*) and averaged from 60°N to 60°S, is –22 W/m^2. (**Bottom**) Average relation between atmospheric pressure and humidity for a 120-year (1870 to 1989) simulation of global warming. The profile is an average of 120 annual mean profiles; the bars represent two standard deviations, indicating that global mean atmospheric relative humidity is conserved over the entire 120-year period. The simulation is from the National Center for Atmospheric Research Community Climate System Model Version 1 (*6*).

period January 1982 to December 2004, to those computed from the temperature and moisture profiles of the Geophysical Fluid Dynamics Laboratory atmospheric GCM, which uses prescribed sea surface temperatures. The temporal trends of the observed and modeled channel 12 observations are in very good agreement, and this agreement persists when the GCM results are repeated with the assumption of constant atmospheric relative humidity. On the other hand, there is considerable disagreement with the channel 12 observations when the GCM results are repeated by assuming no change in the water vapor content of the upper troposphere. Soden *et al.* then use additional satellite observations to emphasize that global mean relative humidity is being conserved by the upper troposphere in response to atmospheric warming.

This work by Soden *et al.* provides the clearest evidence yet that GCMs are properly representing water vapor feedback. This is an important contribution because it eliminates one potential uncertainty within these climate models. There remains, however, an uncertainty in other climate feedback mechanisms, the most notable of which is cloud feedback as described above. The reduction of these uncertainties will require a suite of cleverly designed necessary, but not sufficient, tests of the models.

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CHEMISTRY

Building a Quintuple Bond

Gernot Frenking

Bond order and the division of chemical bonding into single or multiple bonds are among the most fundamental concepts in molecular chemistry. Elements in the main group of the periodic table may have up to three bonds to the same bonding partner (that is, the maximum bond order can only be three). It was long believed that this is the highest bond order that can be achieved in a stable molecule. Because of this conventional wisdom, the 1964 report by Cotton *et al.* (*1*) on the synthesis of a molecule with bond order four caused a sensation. The analysis of transition metal salt compounds containing the anion $[Re_2Cl_8]^{2-}$ revealed a quadruple bond between the rhenium atoms. This finding opened the door to a new field of chemistry and led to the synthesis of a large number of hitherto unknown molecules with multiple bonds having bond orders up to four between transition metal atoms (*2*). It has been speculated that a further extension to bond order five should in principle be possible, but attempts to make a compound with a quintuple bond have been unsuccessful until now. On page 844 of this issue, Nguyen *et al.* (*3*) report the synthesis of a stable compound with fivefold bonding between two chromium atoms (see first figure on the following page).

Chemical bonding between two atoms is usually discussed in terms of bonding and antibonding combinations of the valence atomic orbitals (AOs) that yield molecular orbitals (MOs). The pivotal AOs of the transition metal atoms are the five d orbitals. The figure shows schematically the combination of the d-AOs that give five components for the bonding MOs (σ, π, δ) and five components for the antibonding MOs (σ^*, π^*, δ^*). The diagram also qualitatively indicates the expected ordering for the energy levels of the orbitals. A quintuple bond between two transition metals requires that 10 electrons occupy the lowest lying MOs. This yields one σ bond, one degenerate π bond, and one degenerate δ bond (that is, the π and σ bonds each have two levels with the same energy). Transition metal compounds like $[Re_2Cl_8]^{2-}$ with a quadruple bond have only one (not degenerate) δ bond. Theoretical analysis (*1*) showed that the $d_{x^2-y^2}$ AOs that form the second component of the δ bond (see the figure) interact primarily with ligand orbitals such as the chlorine AOs in $[Re_2Cl_8]^{2-}$. All previous attempts to synthesize a molecule with the general formula $L_nTM\text{-}TML_n$ (where L is ligand, TM is transition metal) in which the $d_{x^2-y^2}$ AOs of TM engage in the "missing" fifth metal-metal bonding rather than in TM-L bonding have failed.

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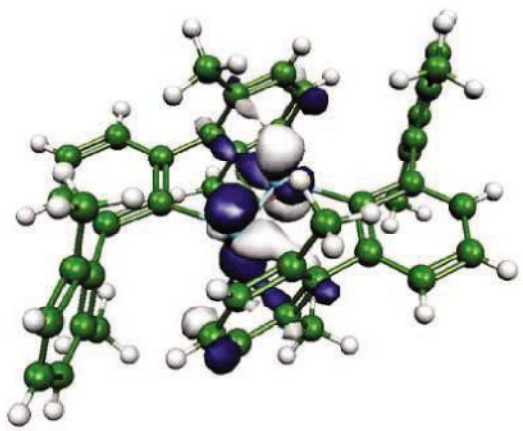
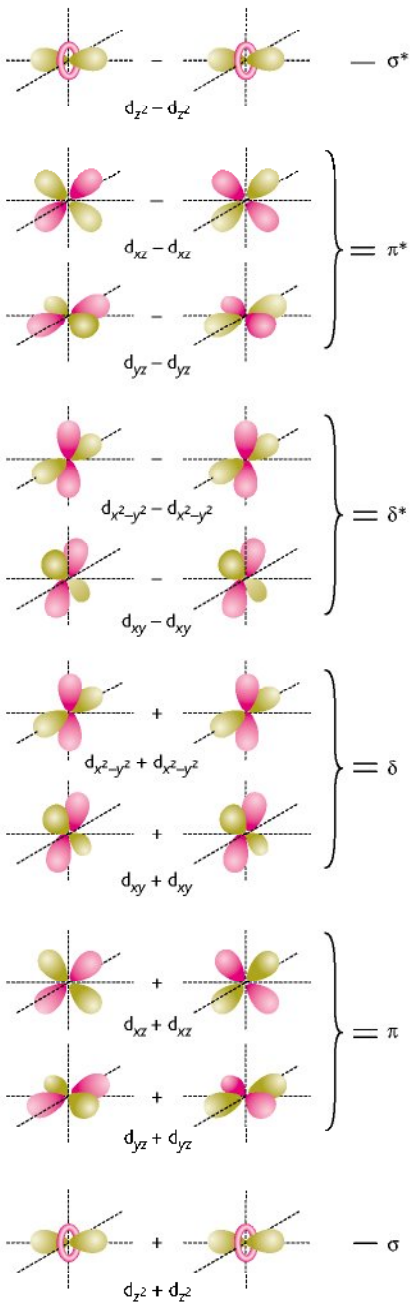
The ligand successfully chosen by Nguyen *et al.* in the synthesis of the quintuply bonded chromium compound Ar′CrCrAr′ is the bulky aryl group Ar′ [C_6H_3-2,6(C_6H_3-2,6-Pr^i_2)$_2$, where Pr^i is isopropyl]. The same ligand has already gained a reputation for stabilizing molecules with multiple bonds that were hitherto not known. A recent example is the synthesis of heavy-atom homologs of substituted acetylenes RC≡CR in which the carbon atoms are replaced by group-14 elements Ge, Sn, or Pb. Power and co-workers succeeded in synthesizing the first examples of the elusive compounds REER with E as Ge (*4*), Sn (*5*), and Pb (*6*), which could even be characterized by x-ray structure analysis when they used the above aryl group in Ar′EEAr′ (*7*). They have now used the same trick for Ar′CrCrAr′. Chromium compounds of the form R_2CrCrR_2 that have a Cr-Cr quadruple bond are known (*2*). Chromium has six electrons for chemical bonding. The difficulty was to find a ligand R that does not attract the electron in the $d_{x^2-y^2}$ AO of the metal but instead allows it to engage in the fifth Cr-Cr bond in a compound RCrCrR. This was achieved with Ar′CrCrAr′ where, in simplified terms, each chromium atom forms one bond to a ligand Ar′ with its s-AO while all five d-AOs are used for the Cr-Cr quintuple bond.



The fivefold way. The Ar′CrCrAr′ molecule synthesized by Nguyen *et al.*, showing the lowest unoccupied molecular orbitals of the chromium-chromium bonds [from (*3*)].

The report by Nguyen *et al.* (*3*) shows that the bonding situation in the compound Ar′CrCrAr′ is more complicated than described above. Some details of the experimental findings will certainly lead to controversial discussions about whether the bonding situation should be considered a true quintuple bond. An obvious argument against this is the finding that the core atoms C-Cr-Cr-C have a trans-bent structure rather than a linear arrangement that should be expected from a genuine quintuple bond. The results of quantum chemical calculations show, however, that there are five occupied MOs in Ar′CrCrAr′, which can be identified as one σ, two π, and two δ Cr-Cr bonds. The π and δ bonds are no longer degenerate because of the lower symmetry of the molecule, but the shape of the MOs resembles that of the classical model even when there is some mixing of the metal-metal and metal-ligand MOs. This explains why the σ orbital is slightly higher in energy than the π orbital. It seems that the bonding situation in Ar′CrCrAr′ is not fully a quintuple bond but rather that there are five components for the metal-metal bonding. A similar situation was found for the germanium and tin analogs of acetylene Ar′EEAr′ (where E is Ge or Sn) (*4*, *5*) and for the related silicon compound (*7*), which are not linear but have a trans-bent geometry. The bending weakens one component of the degenerate π bonding in linear RE≡ER, which means that the bond order is lower than three but still larger than two. The lead compound Ar′PbPbAr′ has also a trans-bent geometry but is a special case where the Pb-Pb bond order is only one (*6*). The bond order in Ar′CrCrAr′ may also be lower than five because of the trans-bent structure, but the very short Cr-Cr bond of only 1.835 Å speaks for a quintuple bond.

Whatever the final conclusion about the bond multiplicity in the newly synthesized compound Ar′CrCrAr′ will be, it is clear that the field of transition metal compounds with multiple bonds has been greatly extended by this work. The authors have paved the way toward synthetic achievements previously considered not to be possible. The door to the experimental field of quintuply bonded molecules has been opened, perhaps not completely yet, but an entrance has been found. This work will inspire others to steer their experimental efforts in a new direction. There is no reason to believe that the structural motif of the quintuple bond in Ar′CrCrAr′ can only be achieved with chromium as the metal atom and with Ar′ as the ligand. Last but not least, the analysis of the bonding situation in the compounds will be a hot topic among theoreticians.



Bonding model. Schematic representation of the chemical bonding between transition metals that is possible with five atomic d orbitals.

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10.1126/science.1120281

ESSAY

EPPENDORF 2005 WINNER

A *Drosophila* OBP Required for Pheromone Signaling

Pingxi Xu

Pest insects have a profound impact on agriculture and human health. Substantial global losses of crops, stored agricultural products, timber, and livestock can be attributed to damage and destruction by insects (*1*). Blood-feeding insects such as mosquitoes, flies, and ticks transmit devastating infectious diseases, including malaria. Insect-borne diseases account for millions of deaths annually, and insect-associated illnesses surpass 300 million annual reported cases (*2*). The toxicity of pesticides and the emergence of pesticide resistance limit their utility. Finding alternative means to control insect pests remains a challenge. Here, I explore a new approach that aims to control species-specific pheromone signaling and thereby modulate insect behavior.

Pheromones are chemical signals emitted by an animal to influence the behavior, physiology, or development of other animals of the same species. In insects, pheromones elicit stereotypic behaviors including mating, reproduction, egg-laying, and aggregation. Understanding the mechanism of pheromone perception could give us the ability to manipulate insect behavior and develop sustainable methods of pest control.

The prevailing theory of pheromone perception is that pheromone-responsive chemosensory neurons are activated directly by pheromone molecules. Indeed, recent work with moth receptors indicates that they can be directly stimulated by pheromone (*3*). However, the pheromone concentrations required to activate heterologously expressed receptors are millions of times the concentrations known to activate insect systems (*4*, *5*). What other components contribute to pheromone sensitivity?

Odorant binding proteins (OBPs) are small, soluble proteins specifically expressed in both olfactory and gustatory systems of terrestrial animals. OBPs are secreted by nonneuronal support cells into the fluid bathing the neuronal dendrites. Members of this class bind directly to odorant ligands. Although the first OBP was identified almost 25 years ago by Vogt and Riddiford (*6*) as a pheromone-binding protein, the in vivo functional significance of these proteins remains elusive. A number of hypotheses have been advanced, including partitioning hydrophobic pheromone from air to aqueous phase, concentrating or sequestering ligands, transporting pheromone to the neuron, or inactivating pheromone (*7*, *8*).

To determine the role of an OBP in vivo, I examined the *Drosophila* mutant, *lush* (*9*), to observe the odorant receptor's neuronal activities and its effects on insect behavior. LUSH (also called OBP76a) is an OBP expressed exclusively in the chemosensory system in both males and females in approximately 150 olfactory hairs (the trichoid sensilla). Trichoid sensilla serve as specialized olfactory structures for pheromone perception in other insects (*10*). Using single-sensillum recording techniques (*11*), I assayed the electrical activity of trichoid olfactory neurons from control and *lush* mutant animals to determine if the mutants showed defects in sensitivity to the only known *Drosophila* volatile pheromone (male-specific), cis-vaccenyl acetate (VA) (*12*–*14*). My work showed that trichoid sensilla from *lush* mutants are completely defective for VA-evoked responses (see the figure, top panel), revealing that the binding protein is required for VA sensitivity (*15*). Expression of a wild-type *lush* transgene in the mutants restores LUSH expression (*9*) and VA sensitivity (see the figure, top panel). Other OBPs, like the moth pheromone binding protein APO3, failed to restore VA sensitivity in the *lush* mutant background, despite its presence in the sensillum lymph in the transgenic animals. Therefore, there is a specific requirement for extracellular LUSH protein in VA sensitivity.

Having established that *lush* mutants are defective for detection of VA, I investigated whether this deficit influences behavior in response to VA pheromone. Because VA is thought to function as an aggregation pheromone that attracts both male and female flies (*12*), I carried out odorant trap assays to test whether *Drosophila* males and females are attracted to VA-producing males or pure VA and whether *lush* mutants are defective for responses to these cues (*9*, *16*). The results show that wild-type male and female flies are equally attracted to wild-type male flies as a source of VA pheromone placed in odor traps. Behavioral attraction of *lush* mutant flies to wild-type males is significantly reduced compared with that of control flies (see the figure, middle panel). When female flies, which do not make VA, were used as bait, I found no difference in this attraction between wild-type and *lush* mutants (see the figure, middle panel). When pure VA was used as bait, both wild-type

Eppendorf and *Science* are pleased to present the prize-winning essay by Pingxi Xu, the 2005 winner of the Eppendorf and *Science* prize for Neurobiology.

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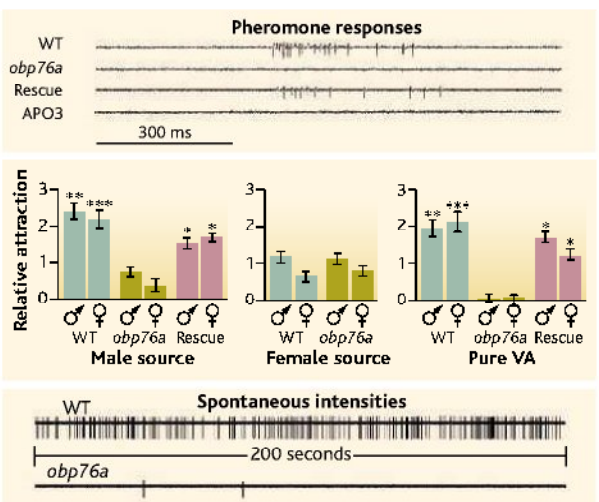

Insensitivity of *lush* mutants to VA. (**Top**) Representative traces of VA-responsive neurons (concentration of VA = 1%). (**Middle**) Behavioral responses of flies to different sources of VA. (**Bottom**) Comparison of spontaneous firing rates of trichoid neurons between wild-type and mutant flies.

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males and females were attracted to it, but mutants lacking LUSH protein were completely defective for attraction (see the figure, middle panel). Accordingly, OBP LUSH is absolutely required for pheromone perception in vivo.

Surprisingly, in addition to the insensitivity to VA, I also noted that the spontaneous activity of trichoid neurons was nearly abolished in the *lush* mutants. Instead of one spike per second, the spontaneous activity in VA-sensitive neurons from *lush* mutants was approximately one spike every 430 ± 55 s, which corresponds to about a 400-fold reduction in spontaneous activity (see the figure, bottom panel). I demonstrated that this defect in neuronal activity in the absence of pheromone is due to loss of LUSH protein and, importantly, recombinant LUSH protein added directly into adult *lush* mutant trichoid sensilla through the recording pipette restored spontaneous as well as VA-evoked responses within 5 min. These data suggest that LUSH is not a simple ligand transporter, but instead may function as an adapter linking the pheromone molecules to neuronal activation, possibly by direct interaction with the receptor or as a coactivator with pheromone. If true, inhibitors of OBPs that prevent pheromone binding might have potential as novel blockers of pheromone signaling.

To determine the feasibility of this approach, I synthesized a series of VA antagonists to target LUSH. The preliminary data show that one of the antagonists can specifically inhibit VA-evoked action potential firing in the wild-type. Conversely, this antagonist induces an excitatory action in the *lush* mutant, but not in the wild-type, suggesting that the inhibitory effect could be LUSH dependent.

In summary, my work provides new insight into pheromone signal transduction and points to new approaches to pest and disease-vector control.

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10.1126/science.1121249

2005 Grand Prize Winner

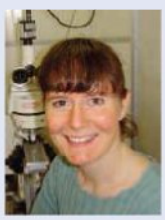
The author of the prize-winning essay, Pingxi Xu, was born and grew up in the northern province of Jiangsu, China. His first career was as a pediatrician, but after years of pediatric practice, Dr. Xu became interested in exploring basic science. To this end, he returned to university in Xian, China, and in 1988 earned a Master's degree in biochemistry and in 1999 a Ph.D. in neurobiology. In 2000 he joined Dr. Dean Smith's lab at the University of Texas Southwestern Medical Center in Dallas. Here he worked hard at understanding the molecular basis of pheromone signaling in *Drosophila*. Dr. Xu's goal is to apply this knowledge to control mosquito breeding by interrupting their perception of pheromones. Although his focus has moved from babies to bugs, his goal remains essentially the same: to improve human health by preventing the occurrence and spread of disease.

Finalists

Justin Blau, for his essay "How Flies Time: Circadian Clocks in *Drosophila*." Dr. Blau was born and raised in London, England. He received his undergraduate degree from Cambridge University in 1991, and his Ph.D. from the Imperial Cancer Research Fund, where he studied basic mechanisms of eukaryotic transcription with David Bentley. After graduating in 1996, he joined Mike Young's lab at the Rockefeller University in New York to study how clock genes drive daily (circadian) rhythms of behavior in *Drosophila*. Dr. Blau started his own lab at New York University in 2000, where he continues to investigate how genes and neurons interact to drive this fundamental behavior.

Paul Frankland, for his essay "Networking to Remember: The Cortex and Remote Memory." Dr. Frankland grew up in Folkestone, England. He studied psychology at the University of Sheffield and completed his Ph.D. work in neuroscience in the lab of Dr. John Yeomans at the University of Toronto. After graduating in 1996, he went on to conduct his postdoctoral work in the lab of Dr. Alcino Silva, first at Cold Spring Harbor Laboratory in New York and then at the University of California, Los Angeles (UCLA). At UCLA he used genetically engineered mice to model normal cognitive function, as well as cognitive dysfunction associated with various inherited diseases. In 2003, he started his own lab at the Hospital for Sick Children in Toronto. A focus in his lab is on understanding how enduring, or remote, memories are organized in the brain.

Johanna Montgomery, for her essay "Synapses in a State: A Molecular Mechanism to Encode Synaptic History and Future Synapse Function." Dr. Montgomery was born and raised in New Zealand. She graduated from the University of Otago in 1999 with a Ph.D. in physiology. During her Ph.D. studies, Dr. Montgomery completed the Neurobiology Course at The Marine Biological Laboratory in Woods Hole, Massachusetts. She began postdoctoral work in the laboratory of Dr. Daniel Madison at Stanford University, where she used paired whole-cell recording techniques to reveal distinct mechanisms of synapse plasticity. She then pursued further postdoctoral training with Dr. Craig Garner at Stanford University to examine the molecular aspects of synapse function. Last year, Dr. Montgomery returned to New Zealand to establish the Synaptic Function Research Group at the University of Auckland, where she is focusing on the molecular and physiological mechanisms of synapse function and plasticity.



For the full text of essays by the finalists and for information about applying for next year's awards, see *Science* Online at www.sciencemag.org/feature/data/prizes/eppendorf/eppenprize.shtml.

2005 Boston, MA FALL MEETING
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SYMPOSIA

ENERGY AND THE ENVIRONMENT

A: The Hydrogen Cycle—Generation, Storage, and Fuel Cells
B: Next-Generation Batteries, Supercapacitors and Other Storage Materials
C: Material Innovations for High-Performance Building Systems
D: Organic and Nanostructured Composite Photovoltaics and Solid-State Lighting
E: Electrochromic Materials and Applications
F: Materials and Technologies for Direct Thermal-to-Electric Energy Conversion
G: Life-Cycle Analysis Tools for "Green" Materials and Process Selection
H: Multifunctional Energetic Materials

BIO-ORGANIC/INORGANIC COMPOSITES

I: Interfaces in Organic and Molecular Electronics II
J: Biomimetic Polymers and Gels
K: Engineering Biointerfaces via Cell-Interactive Materials
L: Mechanical Behavior of Biological and Biomimetic Materials
M: Flexible and Printed Electronics, Photonics, and Biomaterials

NANO- TO MICROSTRUCTURED MATERIALS

N: Dynamics in Small Confining Systems VIII
O: Nanoparticles and Nanostructures in Sensors and Catalysis
P: Quantum Confined Semiconductor Nanostructures—Fabrication, Physical Properties, and Applications
Q: Degradation Processes in Nanostructured Materials
R: Assembly at the Nanoscale—Toward Functional Nanostructured Materials
S: Nanomaterials and the Environment

SMART MATERIALS AND DEVICES

T: Ferroelectric Thin Films XIV
U: Multiferroic Materials
V: Materials and Devices for Smart Systems
W: Electroresponsive Polymers and Their Applications

MECHANICAL BEHAVIOR

Y: Surface Interactions and Surface Engineering for Manufacturing Applications
Z: Amorphous and Nanocrystalline Metals for Structural Applications
AA: Micro- and Nanomechanics of Structural Materials
BB: Mechanisms of Mechanical Deformation in Brittle Materials

ELECTRONICS AND PHOTONICS

CC: Photophysical Properties of Monolayers on Nanomaterials and Surfaces
DD: Materials for Transparent Electronics
EE: Progress in Semiconductor Materials V—Novel Materials and Electronic and Optoelectronic Applications
FF: GaN, AlN, InN, and Related Materials
GG: Plasmonics—Nanoscale Optics and Photonics Based on Metals
HH: Magnetic Sensors and Sensing Systems
II: Fabrication and Characterization Methods for Novel Magnetic Nanostructures

GENERAL INTEREST

X: Frontiers of Materials Research
JJ: Actinides—Basic Science, Applications, and Technology
KK: Solid-Solid Interfaces from Observation to Modeling
LL: Combinatorial Methods and Informatics in Materials Science
MM: *In-Situ* Electron Microscopy of Materials
NN: Scanning Probe Microscopy in Materials Research
OO: Growth, Modification, and Analysis by Ion Beams at the Nanoscale

SPECIAL FORUMS

PP: Forum on Materials Science Education
QQ: IP, TT, VC, IPO, and U

MEETING ACTIVITIES

SYMPOSIUM TUTORIAL PROGRAM

Available only to meeting registrants, the symposium tutorials will concentrate on new, rapidly breaking areas of research.

EXHIBIT AND RESEARCH TOOLS SEMINARS

A major exhibit encompassing the full spectrum of equipment, instrumentation, products, software, publications, and services is scheduled for November 29–December 1 in the Hynes Convention Center, convenient to the technical session rooms. Research Tools Seminars, an educational seminar series that focuses on the scientific basis and practical application of commercially available, state-of-the-art tools, will be held again this fall.

PUBLICATIONS DESK

A full display of over 860 books, plus videotapes and electronic databases, will be available at the MRS Publications Desk.

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Graduate students planning to attend the 2005 MRS Fall Meeting are encouraged to apply for a Symposium Assistant position and/or a Graduate Student Award.

CAREER CENTER

A Career Center for MRS members and meeting attendees will be open Tuesday through Thursday.

The 2005 MRS Fall Meeting will serve as a key forum for discussion of interdisciplinary leading-edge materials research from around the world. Various meeting formats—oral, poster, round-table, forum and workshop sessions—are offered to maximize participation.

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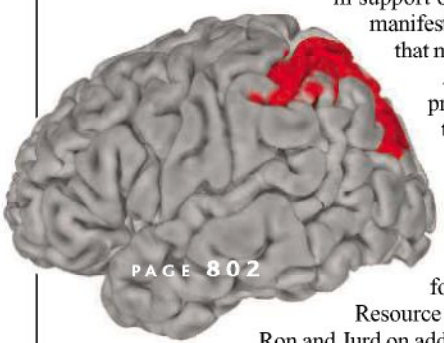
INTRODUCTION

Neuroscience: Systems-Level Brain Development

Our brains show the highest degree of plasticity during the early phases of life. However, not all is lost as we advance in years. A certain level of flexibility and adaptability will be with us throughout life. To fully understand the operations and functions behind these processes, it is not enough to concentrate solely on the molecular and cellular components and their interactions. Nor, at the other end of the spectrum, is the study of higher cognitive functions sufficient: It is often too remote to provide comprehensible mechanistic insight. The leap from cells to thought seems almost infinitely complex, yet every growing child manages to make it. Somewhere in this middle ground, between molecular components and psychology, lie the means by which familial and educational experiences intersect with developmental biology to shape cognitive abilities and personalities. We have thus decided to focus on the systems level instead. This approach has been extremely successful over the years and provided us with a wealth of novel and sometimes astonishing insights.

Sur and Rubenstein (p. 805) set the stage by laying out the framework and controversies within which these questions are presently discussed. They review both the molecular signaling events that underlie early cortical area specification and recent advances in understanding the postnatal shaping of circuits by neuronal activity. Feldman and Brecht (p. 810) describe how certain patterns of sensory activity as well as inactivity elicit multiple, functionally distinct forms of map plasticity in the somatosensory cortex. In a Viewpoint, Sakai (p. 815) discusses the literature on language systems in the mature human brain, with particular emphasis on cortical plasticity during second-language learning. In another Viewpoint, Baron-Cohen *et al.* (p. 819) present data in support of their hypothesis that autism may be an extreme manifestation of the male brain and discuss the mechanisms that might explain these observations.



A News story (p. 802) by Bhattacharjee describes progress in establishing gene-brain-behavior connections in Williams-Beuren syndrome, a neurodevelopmental disorder in which affected individuals are excessively social but typically suffer from mental retardation.

At *Science*'s Signal Transduction Knowledge Environment (STKE, stke.sciencemag.org), the focus shifts to cell and molecular levels. A Teaching Resource by Blitzer on long-term potentiation, a Review by Ron and Jurd on addiction, and a Perspective by Alger on endocannabinoids all address molecular mechanisms of physiological or pathological neuronal plasticity. In other Perspectives in neuroscience, Noon and Lloyd describe a pathway by which the pathogen that causes leprosy subverts the normal response to injury to drive excessive proliferation of Schwann cells.

A Perspective in the Science of Aging Knowledge Environment (SAGE KE, sageke.sciencemag.org) by Jan de Fockert discusses recent results that suggest that cognitive aging is associated with a reduced ability to separate relevant and irrelevant information.

Science's Next Wave profiles stellar early-career neuroscience researchers in North America and Europe, exploring their successes to date. Meanwhile, GrantsNet highlights the latest neuroscience funding opportunities.

–PETER STERN AND PAMELA J. HINES

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See also related Editorial on p. 745;* Science *Express Report by M. Tassabehji* et al.; *SAGE, STKE, and NextWave material on p. 733 or at www.sciencemag.org/sciext/braindev/

Science

NEWS

Friendly Faces and Unusual Minds

Working with a rare set of individuals who have Williams-Beuren syndrome but still show normal intelligence, scientists are trying to tease out what happens in this neurodevelopmental disorder—and shed light on the brain's normal function

To outsiders, a Williams-Beuren Syndrome (WS) convention can seem like a large family reunion. The 200 or so affected individuals who gather for the 3-day biannual event look similar to one another in many ways, although they are not related. Their upturned noses, wide mouths, and small chins give them an elflike appearance—the reason this rare genetic condition, found in 1 out of 7500 people, is also called elfin face syndrome. What's perhaps most striking is the conventioneers' lack of social inhibition. "You walk into the hotel lobby, and they surround you and start talking to you even though you are a perfect stranger," says Karen Berman, a psychiatrist at the National Institute of Mental Health (NIMH) in Bethesda, Maryland.

This excessive friendliness is just one indication that the brains of people with WS work a bit differently from typical brains. In another odd example, WS individuals are incapable of putting together the simplest of puzzles, owing to their inability to visualize an object as a set of parts. That impairment, known as the visuospatial construction deficit, also makes it difficult for them to judge distances and to negotiate stairs. More broadly, even though most people with WS have little difficulty using language and in some cases have notable musical talent, general intelligence tests usually show them to be mentally retarded.

The uniform and well-defined cognitive features shared by those with WS have convinced some researchers that the disorder offers a window into the genetic basis of the human mind. Since the discovery in the early 1990s that the syndrome is caused by the deletion of a tiny section of one copy of chromosome 7, researchers have attempted to identify the roles that the different genes within that section play in the development and functioning of the brain. The broader goal of these efforts has been to learn how cognitive and behavioral features arise from specific genetic traits and their interplay with the environment.

Cognitive window. Most individuals with Williams syndrome share distinctive facial features (*above*) and the same set of physical and mental impairments. The disorder is caused by the deletion of a segment of one copy of chromosome 7, including the *elastin* gene.

These efforts are beginning to pay off. Researchers have drawn links between the genes absent in WS, structural and functional abnormalities in certain brain regions, and cognitive deficits that are the hallmarks of the disorder. Some of the gene-brain-behavior links have subsequently been confirmed in mouse models, and scientists have uncovered neurodevelopmental pathways that are disrupted by the deletion of WS genes. Taken together, these findings "have been invaluable in understanding how relatively subtle developmental defects can have a significant impact on neurological function," says Dennis O'Leary, a neurobiologist at the Salk Institute for Biological Studies in San Diego, California. The work, he adds, opens the door to explaining how genes work through the brain to make us who we are.

The neural connection

Although other physicians may have come across earlier cases of the disorder, British physician J. Williams was the first to identify it in a 1961 paper that described children with a unique set of facial, cognitive, and heart defects. A second research group, led by German cardiologst Alois J. Beuren, independently identified the syndrome the following year, adding excessively social behavior to its list of characteristics.

As a step toward understanding how genes contribute to the cognitive profile in WS, researchers have sought to determine the neural mechanisms that underlie signature traits of the illness. One challenge they have faced is the mental retardation of most people with WS, which makes it difficult to perform many experimental tasks testing cognition. Karen Berman, along with NIMH neurologist Andreas Meyer-Lindenberg, psychologist Carolyn Mervis of the University of Louisville, Kentucky, and others, got around that hurdle by assembling from around the world 13 volunteers with WS who had both the chromosomal deletion and the cognitive deficits characteristic of the syndrome but showed normal overall intelligence.

In one set of experiments, the researchers had the volunteers perform two tasks aimed at elucidating the visuospatial construction deficit. In the first, they asked the individuals whether two pieces of a puzzle presented on a computer screen could fit together to form a square. In the second, volunteers had to determine whether images presented one after the other were located at the same height on the screen. Comparing the functional magnetic resonance images (fMRI) of the WS group with those of healthy controls, the researchers found that the WS individuals showed significantly lower neuronal activity in a part of the brain used by the spatial processing pathway of the visual system.

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In contrast, the people with WS showed normal brain activity along the neural pathway responsible for identifying objects, which may explain why they seem to have little difficulty in recognizing faces or other visual material.

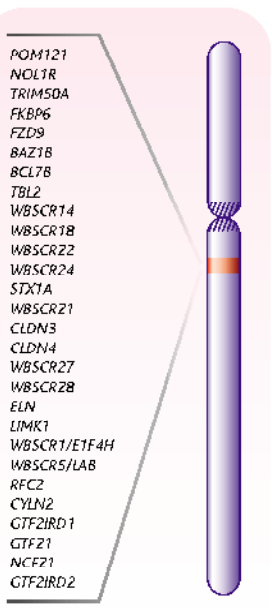
Using MRI scans to examine structural details of WS-affected brains, the researchers found an abnormally low density of nerve tissue adjacent to areas where activation was weak during the two tasks, suggesting that this region was not contributing its fair share of input to the spatial processing stream. This anatomical flaw—in the fold separating the parietal and occipital lobes (parietooccipital sulcus)—was a likely basis for the visuospatial construction problem in WS patients, Berman and her colleagues concluded last year in a report in *Neuron*. The researchers have now followed up by analyzing the geometry of the fold; they reported in the 24 August *Journal of Neuroscience* that it was significantly shallower in the WS volunteers than in controls. And in the 1 July *Journal of Clinical Investigation*, the group reported other studies on the same set of patients that revealed structural and functional abnormalities in the hippocampal region, which offers a possible explanation for long-term memory impairments and other cognitive deficits in WS.

To some WS researchers, the normal intelligence of the volunteers in the NIMH-led studies presents a problem. "What's vexing is that their IQ makes them unrepresentative of the general population of WS patients, and yet that very feature makes them good experimental subjects," says Allan Reiss, a psychiatrist at the Stanford University School of Medicine.

Meyer-Lindenberg rejects such skepticism. The WS people his team recruited showed the same visual deficits as mentally retarded WS patients, which means they were not able to circumvent their defective neural mechanisms while performing the assigned tasks. "If we'd had a negative finding—that is, if the volunteers had performed as well as the controls, we could have suspected that their intelligence was helping them to somehow compensate for their handicap. But to find eye-popping abnormalities and still ascribe that to the IQ difference between them and the general WS population, we'd have to make up some very convoluted reasoning," he says.

Despite this disagreement, Reiss and his colleagues have come up with some of the same results. In one experiment, Reiss's team compared brain scans of 43 WS individuals with characteristically low IQs to those of 40 healthy subjects and found low densities of nerve tissue in certain regions along the spatial processing pathway. In another study, the researchers looked at fMRI scans of 11 patients who were asked to determine whether faces presented on a computer screen were gazing at or away from them. (This was a simpler task than the ones used by Berman's group.) Not only were the people with WS slower in their responses than controls, but they also showed significantly less activity in their primary and secondary visual cortices while performing the task, Reiss and his colleagues reported in *Neurology* last year.

Decoding the brain. NIMH's Karen Berman and Andreas Meyer-Lindenberg are studying 13 WS individuals with normal intelligence.



Missing. In all, 28 genes have been identified in the chromosome 7 region deleted in typical WS cases.

A faulty template

Pinpointing the neural underpinnings of cognitive deficits in WS is only one piece of the puzzle. Another is linking genes to those anatomical and functional defects. Even though the chromosomal deletion in WS encompasses just 28 known genes—a very small number given that thousands of genes are involved in brain development—isolating their specific contributions to the cognitive aspects of the disorder is a complex problem. "These genes could be interacting among themselves and with other genes in a ridiculous number of ways," says Julia Korenberg, a molecular geneticist at the Cedars Sinai Health System in Los Angeles, California.

Researchers have attempted to narrow the list by studying a few people who have shorter deletions on chromosome 7 than is seen in individuals with WS and yet show some of the same cognitive characteristics. For example, in a study published online by *Science* this week (www.sciencemag.org/cgi/content/abstract/1116142), a British-American team led by May Tassabehji, a medical geneticist at the University of Manchester, U.K., adds to the evidence that a gene called *GTF2IRD1* plays a role in the visuospatial deficit. The researchers identified a 4.5-year-old girl with a chromosome 7 deletion that included this gene but excluded many of the other candidates. The report centers on how the gene's loss may explain the girl's WS-like facial features, but the researchers note that she also has serious problems with spatial navigation

In some of the earliest work using this partial-deletion strategy, reported in 1996, Mervis and geneticist Colleen Morris of the University of Nevada, Las Vegas, identified a gene called *LIM kinase 1* as a strong candidate to explain the visuospatial construction deficit. (The group also used the technique to identify a gene that codes for elastin as a contributor to the vascular and heart defects in WS.) But the *LIM kinase 1* story is confusing: Researchers have identified individuals missing one copy of the gene who show none of the WS cognitive defects.

Studies in recent years have implicated other genes within the cluster of 21 for the visual deficit, two prominent ones being *GTF2IRD1* and *Gtf2i*, both identified by Korenberg in collaboration with the Salk Institute's Ursula Bellugi and others. Findings from other partial-deletion cases have thrown two more genes to the mix: *frizzled 9* and *cyclin2*.

Mouse models are helping sort out the roles of the different candidates. In work reported in *Neuron* 3 years ago, for example, Zhengping Jia of the University of Toronto in Canada and his colleagues knocked out the *LIM kinase 1* gene in mice and demonstrated

that the animals had poor synaptic function and memory. Neurons in these mice had inadequate dendritic spines, the protruding tendrils on the surface of a nerve cell that help form excitatory synapses.

And in experiments described in the June issue of *Development*, clinical neurologist Samuel Pleasure of the University of California, San Francisco, and his colleagues found that mice lacking one or both copies of the *frizzled 9* gene ended up with fewer-than-normal neurons in their hippocampus, due to a surge in programmed cell death in that region. The gene defect significantly hampered the animals' spatial learning abilities.

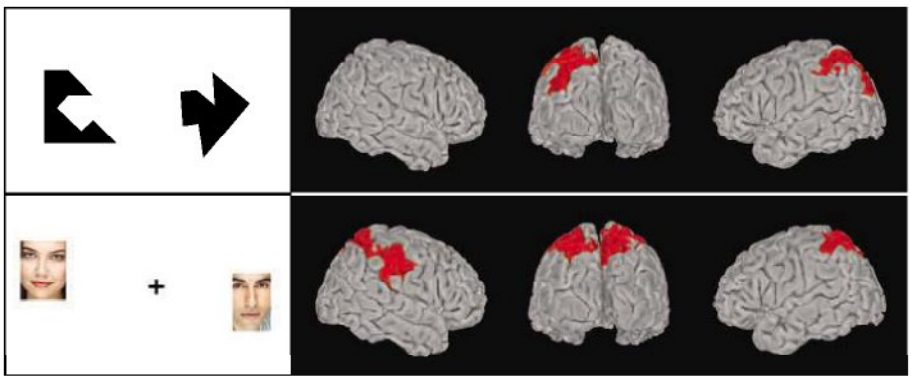
Brain autopsies of WS patients are also shedding light on the disorder's visual problem. Surveying the molecular landscape of one such brain, Harvard neurologist Albert Galaburda and his colleagues found an abnormally low expression of *Gtf2i* in the peripheral visual cortex and superior parietal regions. In earlier WS autopsies, the same group had discovered that the neurons in the dorsal parietal cortex—a part of the spatial processing system—were larger and stubbier than normal, suggesting that they had not been patterned correctly during the brain's development. "It's possible that *Gtf2i* lies in the pathway of certain dorsal patterning genes, and its low expression is selectively detrimental to neuronal development in the dorsal parietal cortex," speculates Galaburda, whose group presented the work at the Society for Neuroscience meeting last year.

So which of these half-dozen genes actually underlies the syndrome's visuospatial construction deficit? "I don't think anybody would want to get into a contest about whose gene is more important," says Pleasure. "The likely scenario is that multiple genes are responsible. This may be a more well-defined syndrome than other genetic disorders, but it's still quite complicated."

Afraid of none

A video clip running on Berman's desktop computer provides a vivid illustration of the excessively social nature of people with WS. The video shows an 18-month-old girl with the disorder interacting with a normal 5-year-old boy who's sitting on the floor. She walks up to within a few inches of him and peers into his face with great intensity. When the boy starts to get uncomfortable after a few seconds and turns his head, she shifts position to continue staring at him from up close. Even after he stands up and begins bouncing a basketball on the floor, she doesn't relent.

Despite such social fearlessness, WS patients typically display high levels of nonsocial anxiety, such as fear of heights. Berman and her colleagues have sought to tease apart the neural basis of this paradoxical behavior by asking their normal-IQ WS volunteers to perform two tasks. In the first, the researchers presented them with an image of a face showing anger or fear and, a few seconds later, two other faces simultaneously. They were then asked to pick which of the latter faces bore the same emotion as the first. The second task required a similar kind of matching—only, instead of faces, the images presented on the computer were of fear-provoking scenes such as a boat sinking or a house burning. As a control task, the volunteers had to match one of two geometrical shapes to a shape shown earlier.



Spatial challenge. While performing a square-completion task (*top*) and a location task (*bottom*) in the NIMH-led study, individuals with WS showed lower than normal activity (red) in brain regions lying along the spatial processing pathway.

Comparing fMRI scans taken during these tasks, the researchers found significant differences between the WS group and a control group in the activation of the amygdala, a brain region known to regulate people's fear response. For the task involving threatening faces, the amygdala in the WS individuals was much less active. In contrast, while performing the second task, using scenes rather than faces, these volunteers showed higher amygdala activation than did the controls. The researchers also found that during either task, the orbitofrontal cortex (OFC) was less active among the people with WS than in controls, while the medial prefrontal cortex (MPFC) was more active. Berman says the findings, reported in the August issue of *Nature Neuroscience*, fit nicely into a model of social cognition in which amygdala function—and therefore fear response—is regulated by both the OFC and MPFC. She notes that her group has documented a structural abnormality in the OFCs of WS individuals, which may explain their low fear response to faces.

A complete account of the cognitive problems in WS must include the role of the environment in mediating the syndrome's effects, researchers stress. That role could be especially important for social cognition, says Ralph Adolph, a cognitive neuroscientist at the California Institute of Technology in Pasadena. "Since the genes influence social behavior very early on in WS individuals, their unusual social behavior in turn is likely to construct an abnormal social environment—that is, other people will socially interact with a WS child differently than with a child without the syndrome," he says. "I think we can certainly draw a link between genes and cognition, as long as we realize that the link is very complex and always brings in the environment in its mediation."

Evidence that more than genes governs the cognitive abilities of those with WS comes from findings that "individuals with the same classic WS deletion vary considerably in their visuospatial construction ability, although almost all show a significant deficit," says Louisville's Mervis. "On average, individuals who have a parent who is good at drawing are themselves better at drawing than are other individuals with the same deletion; this is likely due to a transaction between genes from outside the deleted region and the environment. Children in these families may well have more opportunities to draw, in addition to having better adult models of how to draw."

Nobody expects that there's a simple, straight line connecting genes to the mind, says Reiss, who along with his colleagues is planning a longitudinal study of children with WS. Such work, he hopes, will shed light on both the genetic and environmental pieces of the puzzle. "We have the possibility of unraveling how genes and environmental moderators shape cognition and behavior," he says. "Now that is really exciting stuff."

—YUDHIJIT BHATTACHARJEE

Patterning and Plasticity of the Cerebral Cortex

Mriganka Sur[1]* and John L. R. Rubenstein[2]*

The cerebral cortex of the human brain is a sheet of about 10 billion neurons divided into discrete subdivisions or areas that process particular aspects of sensation, movement, and cognition. Recent evidence has begun to transform our understanding of how cortical areas form, make specific connections with other brain regions, develop unique processing networks, and adapt to changes in inputs.

The degree to which our genetic endowment (nature) versus our experiences (nurture) mold the development and function of our brains has been the subject of robust discussion and experimental investigation. Research before 1990 led to two general hypotheses: the Protomap (*1*) and the Protocortex (*2*). In their most extreme interpretations, the former postulated that the cortical progenitor zone contains the information that generates cortical areas, whereas the latter postulated that thalamic afferent axons, through activity-dependent mechanisms, impose cortical areal identity on an otherwise homogeneous cortex. In the intervening 15 years, tremendous strides have been made in understanding cortical development with molecular, genetic, imaging, and electrophysiological approaches. The new evidence indicates that the development of cortical areas involves a rich array of signals, with considerable interplay between mechanisms intrinsic to cortical progenitors and neurons and mechanisms extrinsic to the cortex, including those requiring neural activity.

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Early Patterning of the Cortex

Early development of the cortex is highly integrated with development of other parts of the brain, including midline patterning centers, the basal ganglia primordia that produce many of the cortical local circuit neurons, and axonal inputs from the thalamus and brain stem. The cortex, more generally known as the pallium, develops from a morphologically uniform ventricular zone located in the dorsocaudal part of the telencephalic vesicles. The pallium is further subdivided into medial pallium (MP), dorsal pallium (DP), lateral pallium (LP), and ventral pallium (VP), which will respectively give rise to the hippocampal formation (limbic lobe), the neocortex, the olfactory/piriform cortex, and the claustrum and parts of the amygdala (*3*, *4*). Each of these large domains is divided into subdomains, such as the functional subdivisions (areas) of the neocortex.

Mature cortical areas differ by their location within the cortex, molecular properties, histological organization, patterns of connectivity, and function. Within the neocortex, rostral regions regulate motor and executive functions, whereas caudal regions process somatosensory, auditory, and visual inputs. These different cortical areas have a precise connectivity, particularly with nuclei within the dorsal thalamus, which provides some of the principal inputs to the cerebral cortex (Fig. 1).

Programs of regional identity and morphogenesis in the pallium are directed in part by signaling centers producing secreted molecules. These centers are initially located along the edges and midline of the neural plate and later along and flanking the midline of the telencephalic vesicles (Fig. 2A) (*3*, *5*–*8*). Sonic hedgehog (Shh) is expressed in the ventral telencephalon and hypothalamus. Shh is essential to the regionalization of the subpallium and also regulates morphogenesis and patterning of the pallium (*9*–*11*). Along the dorsal midline, secreted molecules of the bone morphogenetic protein (Bmp) and Wnt families control patterning of the medial and dorsal pallium, including the hippocampus and neocortex and the choroid plexus (*12*, *13*). The targeted inactivation of Wnt3a, or of the Wnt signaling factor Lef-1, severely disrupts the formation of the hippocampus (*14*, *15*). Ectopic expression and reduction of Bmp signaling alters development of the dorsal midline and paramedial structures (i.e., choroid plexus)

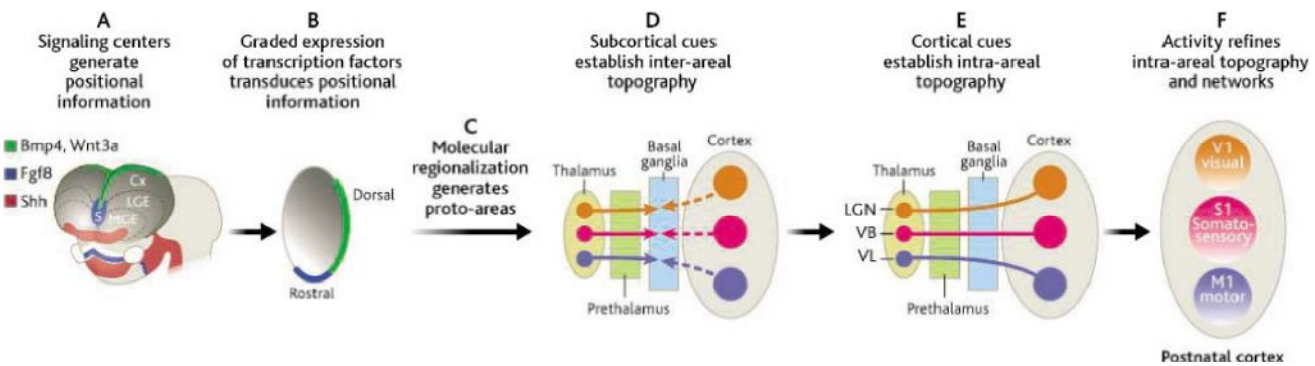

Fig. 1. Steps in generating cortical areas. (**A**) Secreted proteins from patterning centers generate positional information. (**B**) Gradients of transcription factor (such as Emx2) expression are created in the cortical ventricular zone, which (**C**) confer specific regional identities to its derivative, the cortical plate, forming proto-areas. (**D**) The topography of axons growing from the cortical plate and thalamus is regulated by intermediate target zones (basal ganglia and prethalamus); this generates a coarse inter-areal projection map. (**E**) Upon arrival of the axons to their target zones, low-resolution intra-areal connections are generated. (**F**) Thalamic and cortical connections are refined by activity-dependent mechanisms to generate mature intra-areal maps and networks. Cx, cortex; LGE, lateral ganglionic eminence (striatum); LGN, lateral geniculate nucleus; MGE, medial ganglionic eminence (pallidum); S, septum; VB, ventrobasal thalamus; VL, ventrolateral thalamus. Adapted from (*3*).

(*16*) and also affects patterning of the dorsal pallium (*9*, *13*, *17*, *18*).

At the rostral margin of the telencephalon, a source of Fgf8 promotes telencephalic outgrowth and regulates its rostral regionalization (*5*, *8*, *19*–*23*). Fgf8 expression in the rostral telencephalon is nested within the expression of other Fgfs (*24*). Mutations in the mouse and zebrafish Fgf8 gene and experimental manipulations of Fgf8 function have demonstrated its dosage-dependent functions, including regulation of the size and nature of the frontal cortex, telencephalic midline structures, and basal ganglia (*19*–*21*, *23*, *25*–*28*). The phenotypic complexity reflects, in part, reciprocal interactions between the patterning centers. For instance, Bmp4 expression and apoptosis decrease in the rostral midline of Fgf8 hypomorphs, whereas they increase when Fgf8 expression approaches the null state (*23*). Thus, the level of Fgf8 regulates the level of Bmp4 expression in a nonmonotonic manner, which in turn leads to different effects on the patterning and survival of dorsal midline cells as a function of Fgf8 dosage, perhaps through expression of Sprouty (*29*), an Fgf8-induced repressor of receptor tyrosine kinase signaling (Fig. 2B).

An intriguing concept that arises from these studies is that alterations in the relative strengths of the patterning centers would lead to modifications in the relative sizes of different cortical regions. For instance, an animal with a weak Fgf-patterning center would be expected to have a relatively small prefrontal cortex and therefore might exhibit a cognitive profile found in "hypofrontal" patients. Ectopic Fgf8 expression can duplicate somatosensory cortex, which shows that it can specify regional fate and suggests a mechanism for how new cortical areas could evolve (*19*, *30*).

The patterning centers operate in part through generating graded expression of the transcription factors that control histogenetic programs for proliferation, neurogenesis, migration, connectivity, and cell death/survival. Several genes encoding transcription factors, including Foxg1 (BF1), COUPTF1, Emx2, Lef1, Lhx2, and Pax6, are expressed in gradients along the mediolateral (dorsoventral) and rostrocaudal axes of the cerebral cortex (*15*, *31*–*37*). Alteration in Fgf8 signaling regulates the expression of several transcription factors (Fig. 2B); for instance, Fgf8 increases Foxg1 (BF1) expression and reduces Emx2 and COUPTF1 expression (*5*, *20*, *22*, *28*, *38*). Analysis of mice lacking COUPTF1, Emx2, Foxg1, Gli3, Lhx2, Pax6, and Tlx demonstrates that these transcription factors are essential for regionalization of the cortex. Progenitors in Foxg1 and Lhx2 mutants exhibit expansion of dorsalmost molecular features (Bmp and Wnt expression) (*37*, *39*, *40*); Emx2 and Pax6 also regulate expression from the patterning centers (*8*, *28*, *36*). Foxg1 inhibits differentiation and promotes proliferation (*37*, *40*). Gli3 represses ventral fates (*10*, *11*, *41*, *42*). Pax6 and Tlx regulate the formation of the ventral pallium and its boundaries (*43*–*45*). Pax6 is also involved in specifying rostral identity (*32*), and COUPTF1 in regulating caudoventral identity (*46*, *47*).

In Emx2 $^{-/-}$ neonatal mice, the occipital cortex (presumptive visual cortex) adopts molecular fate characteristics of the parietal neocortex (presumptive somatosensory cortex) (*33*, *37*, *47*, *48*). This molecular shift correlates with a corresponding shift in thalamic projections, raising the possibility that molecular properties and the targeting of thalamic axons are controlled by Emx2 expression. Subtle increases or decreases in Emx2 expression lead to expansions or contractions in the relative sizes of neocortical subdivisions (*37*, *46*, *47*, *49*–*51*).

The programs of regionalization are linked to the programs of neurogenesis, which influence laminar identity and therefore the input and output projections of a cortical area. The prevailing model is that cortical glutamatergic projection neurons of the subplate and layers 6 to 2 are produced sequentially by the cortical progenitors (*1*), cortical GABAergic (γ-aminobutyric acid) local circuit neurons are produced by the subpallial progenitors (*52*), and Cajal Retzius cells of layer 1 are produced in the dorsal-most pallium (*37*). The subplate and layer 6 send projections to the thalamus; layer 5 sends projections to the basal ganglia as well as to the thalamus, midbrain, and brain stem; layer 4 receives projections from the thalamus; and layers 2 and 3 send projections to other cortical areas. Progress is just beginning to be made in the molecular specification of cortical laminar identity (*53*, *54*). A critical unanswered question is how the gradients of transcription factor expression in cortical progenitors are translated into discontinuous molecular features within cortical layers; this process is probably linked to the development of region-specific connectivity maps.

Cortical Arealization and Axon Guidance

Emx2, Pax6, and COUPTF1 regulate the restricted expression of genes in a region-specific and layer-specific pattern (*33*, *46*–*48*, *55*). These genes encode transcription factors (Id2, RZR-beta, and Tbr1), adhesion molecules (Cadherin 6 and 8), and axon guidance molecules (EphrinA5) and reflect the parcellation of the cortical plate at birth. Thus, these molecules could participate in regulating axonal connections and influence thalamocortical, intracortical, and corticothalamic connectivity. For instance, molecular signals in the cortex regulate the pattern of inputs from thalamic nuclei, as illustrated in EphrinA5 mutants (*56*) and by overexpression of Fgf8 (*19*).

Fgf8 hypomorphic mutants have a profound disruption in the pattern of neonatal intraneocortical projections, which shows that signals from the rostral cortex have an important role in restricting the growth of rostral projections from neurons in the caudal cortex (*57*). However, no defect in reciprocal topographic projections between immature cortical areas and thalamic nuclei is observed in these neonatal Fgf8 mutants, despite molecular reorganization of rostral cortex (*20*). The latter result suggests that establishment of the early topography of thalamocortical projections is regulated primarily by signals that the growing thalamic afferents sense during their pathfinding through the diencephalon and subcortical telencephalon.

Several lines of evidence now support the model that signals in the diencephalon and subcortical telencephalon regulate the location where axons from a given thalamic nucleus enter the developing cortex (for example, that visual thalamus axons enter the caudal cortex,

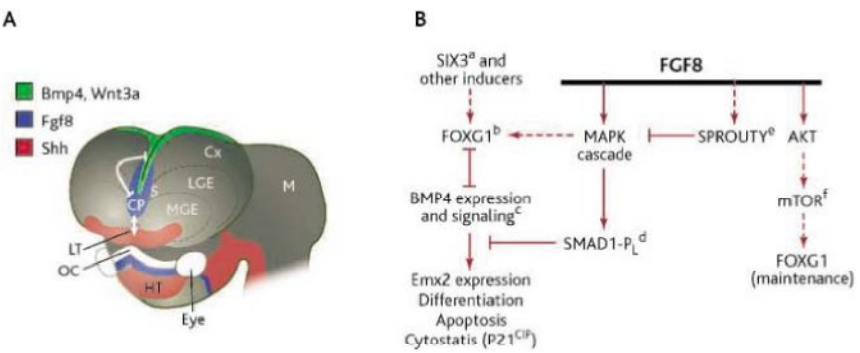

Fig. 2. (**A**) Rostrolateral view of a mouse brain at about 10.5 embryonic days, highlighting the patterning centers. White arrows, positive interactions; white lines with bars, repressive interactions (*23*); CP, commissural plate; Cx, Cortex; HT, hypothalamus; LT, lamina terminalis; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; M, mesencephalon; OC, optic chiasm. (**B**) Selected signaling cascades downstream of Fgf8 that regulate expression of key transcription factors Emx2 and Foxg1. Dotted lines indicate indirect steps. Not shown: Fgf8 represses Wnt expression. For steps a, b, c, d, e, and f, see, respectively, (*137*), (*40*), (*138*), (*139*), (*29*), and (*140*).

whereas motor thalamus axons enter the rostral cortex) (*58*). For instance, mice with defects in the basal ganglia (Ebf1), or the basal ganglia and prethalamus (Dlx1/2), have systematic errors in the topography of thalamic projections to the prenatal cortex; their immature somatosensory thalamus projects axons to the region of the immature visual cortex (*3*, *59*, *60*). Gradients of EphrinA5 in the basal ganglia and EphA4 in the thalamus contribute key molecular cues for organizing this topography (*61*).

The growth of thalamic axons to the cortex appears to be influenced by axons growing from the cortex to the thalamus. Mice with a paucity of normal corticothalamic axons (Tbr1 mutants) show a defect in the growth of thalamic axons to the cortex (*53*). This and related findings are consistent with some of the postulates of the "handshake hypothesis" (*58*) and with the proposal that the topography of thalamocortical axons is regulated in part by interactions with their environment during development (Fig. 1). Furthermore, thalamic afferents grow through cortical progenitors in the subventricular zone, where the axons have been postulated to regulate the cell-cycle kinetics that contribute to histological differences between primate visual areas 17 and 18 (*62*).

Specificity of Thalamocortical and Intracortical Connections

Innervation of cortical areas by thalamic axons is the first step in creating processing circuits within the cortex (*58*). Subsequently, interlaminar connections between cortical neurons within a column, together with local and long-range connections, form the major networks that are characteristic of a cortical area and that transform inputs into outputs that are conveyed to other cortical or subcortical structures.

An important step in innervation of the cortex by thalamic axons is innervation of the deepest cortical layer, the subplate (*63*, *64*). In the subplate, thalamic axons "wait" for cortical layer 4 neurons to migrate and settle before entering the cortical plate and making contact with these target cells (*65*, *66*). Ablation of the subplate prevents the formation of ocular dominance columns (*67*) as well as orientation-selective cells and orientation columns (*68*). More generally, signals in both the subplate and the cortical plate have a role in directing the growth of thalamic axons, as shown in experiments in which an ectopic somatosensory cortex is generated by ectopic Fgf8 (*30*).

The cortex has remarkably specific interlaminar and intralaminar connections between individual cells and cell classes (*69*–*73*). Layer 5 neurons in somatosensory or visual cortex receive interlaminar inputs from specific cells (*74*), form stereotyped local connections (*75*), and develop specific early long-distance projections (*76*). A subset of strong intracortical connections exists within a set of weaker connections, the latter providing a potential substrate for synaptic strengthening and network plasticity (*77*). Fine-scale connections and specific sparse connections characterize interlaminar networks (*72*, *73*). Positional cues within individual cortical layers regulate the formation of layer-specific projections, probably through diffusible or membrane-associated molecules (*78*). Semaphorin/neuropilin and ephrin/eph ligand/receptor proteins are implicated in directing specific aspects of axonal and dendritic growth. For example, semaphorin 3a is expressed in the superficial cortical plate, where it can repulse pyramidal cell axons (*79*) and attract their apical dendrites (*80*).

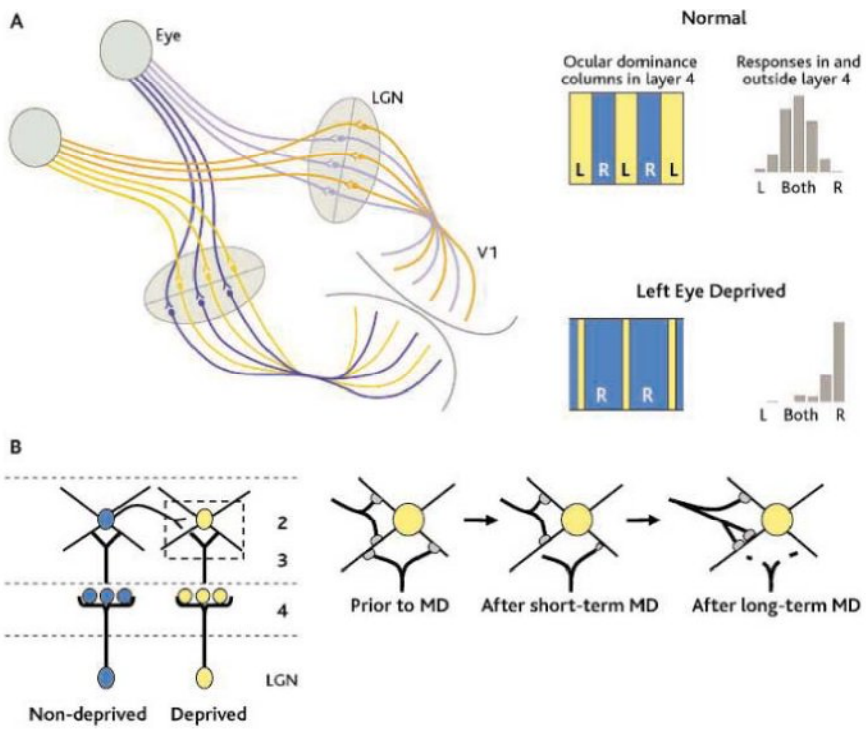

Fig. 3. Activity shapes connections in visual cortex. (**A**) Projections from the two retinas are targeted to the thalamic lateral geniculate nucleus (LGN) and subsequently to the primary visual cortex (V1). In higher mammals, the projections form alternating columns within layer 4, representing inputs from the right and left eyes, respectively. Suturing one eye shrinks its columns and causes cortical cells to respond nearly exclusively to the open eye. Adapted from (*91*, *92*). (**B**) Functional and structural changes after monocular deprivation occur rapidly in the superficial and deep layers of cortex (*109*). The first changes are a reduction in size and loss of spines (gray ovals) driven by the deprived eye (*107*). Deprivation for a longer period causes a more significant loss of spines driven by the deprived eye (*110*), shrinkage of deprived eye axon arbors (dashed line connecting upward), and an expansion of connections from the nondeprived eye. These intracortical changes likely precede similar changes in thalamocortical connections, shown in (A).

Activity-Dependent Mechanisms of Network Formation and Plasticity

A scaffold of cell-specific connections and hence of area-specific networks may be laid down by molecular recognition and adhesion mechanisms, perhaps using splice variants of cell adhesion proteins such as cadherins (*81*) and neurexins (*82*). Superimposed on this framework, activity-dependent mechanisms shape connections between neurons.

Spontaneous electrical activity is present at the earliest stages of cortical development, and stimulus-driven activity in sensory pathways is particularly prominent at later stages of development. Electrical activity can be permissive, to trigger molecular or developmental programs that create connections, or instructive, to shape particular connections or their strength. Activity operates through modulating the expression and function of almost the entire range of molecules responsible for neuronal and synaptic function [see, for example, (*83*)]. Activity-dependent regulation of synaptic strength (plasticity) may be a manifestation of intrinsic homeostatic mechanisms to preserve a particular level of synaptic drive (*84*). Thus, for later stages in cortical development, the line between "activity-independent" and "activity-dependent" mechanisms of development is increasingly becoming blurred. Intracortical connections, which form later than

thalamocortical connections, also exhibit protracted plasticity (*85*, *86*).

Studies that alter inputs to primary sensory cortices have been fundamental for understanding activity-dependent mechanisms that regulate development of neuronal circuits. For brevity, here we will focus on two components of the primary visual cortex (V1): ocular dominance columns and orientation columns. The formation of these circuits illustrates the interplay between early developmental scaffolds and later patterned electrical activity in shaping local and long-range (tangential) intracortical networks.

Ocular dominance columns in V1—domains driven by either the left or right eye—arise by the segregation of retinal inputs relayed through eye-specific layers of the lateral geniculate nucleus (Fig. 3A, left). The initial ingrowth of axons into visual cortex from eye-specific thalamic layers targets eye-specific cortical domains (*87*–*89*), which suggests the existence of molecular cues that underlie the formation of ocular dominance columns. However, activity-dependent mechanisms are required to consolidate the patterning and establish the connectivity of eye-specific projections. Suturing the lids of one eye during a critical period early in life reduces the size of ocular dominance columns related to the sutured eye (Fig. 3A, right) (*90*–*92*). These experiments demonstrate ocular dominance plasticity, whereby the open or untreated eye dominates the cortex both physiologically and anatomically, presumably through competition between axons from the two eyes for cortical territory and synaptic linkage with target cells.

Ocular dominance plasticity is also shaped by the activity of local circuit neurons. Basket cells, GABAergic inhibitory neurons with widespread axonal collaterals, are critical for mediating the plasticity, through synapses containing alpha1 GABAergic receptors on pyramidal cells (*93*). Similarly, modulating GABAergic inhibition in visual cortex of cats alters the spacing and periodicity of ocular dominance columns (*94*): Enhancing inhibition increases ocular dominance column width. Thus, these studies implicate lateral inhibitory interactions in specific aspects of functional connectivity and structural patterning within visual cortex.

Cortical cells and networks seek to preserve a balance between excitation and inhibition as they develop synaptic connections (*95*). A critical level of inhibition is required for initiating and terminating ocular dominance plasticity (*96*); disrupting the balance by even subtle manipulation of inhibition alters functional and structural connections within cortical networks. Similarly, cortical neurons seek to maintain a given level of synaptic drive by scaling their inputs in homeostatic fashion (*97*). The effects of various manipulations of visual activity, including monocular deprivation and dark-rearing, can be understood within the framework of these mechanisms that operate during normal development to organize cortical connections (*84*, *96*).

How might electrical activity influence the structural organization of cortical connections? A critical locus of both physiological and anatomical change is at the level of dendritic spines, which are structural specializations that contain the postsynaptic elements of excitatory synapses [for a review, see (*98*)]. In vitro, long-term potentiation is correlated with enlargement of spines and addition of new spines (*99*, *100*), whereas long-term depression is correlated with shrinkage of spines (*101*); these shape changes are mediated in part through the degree of actin polymerization (*102*, *103*). In vivo, spine numbers on somatosensory cortex neurons are reduced if their afferent inputs are removed either during development or during adulthood (*104*, *105*). Thus, reduction of synaptic drive may be sufficient to destabilize and reorganize spine structure and number.

Activity rapidly alters dendritic spines (*106*, *107*) and synaptic connections (*108*, *109*) in V1 during development, particularly outside layer 4. Spines show increased structural motility and a reduction in size following brief monocular deprivation (Fig. 3B), likely as a prelude to the reduction of connections from the deprived eye after longer visual deprivation (*110*). A number of signaling molecules that may function as a cascade, including Ca^{++}/calmodulin-dependent protein kinase II (CaMKII), cAMP-dependent protein kinase (PKA), and extracellular signal–regulated kinase (ERK), intervene between synaptic activation, calcium entry, actin polymerization, and reorganization of synaptic connections (*96*, *111*–*113*). A key mechanism underlying enhanced spine dynamics and spine loss following unbalanced visual activity is proteolysis of the extracellular matrix by the serine protease, tissue plasminogen activator (tPA) (*107*, *112*).

Orientation selectivity in V1 is a second system for examining the role of activity in the development of visual cortex networks. Orientation-selective neurons in V1 respond best to edges of light of a particular orientation placed within their receptive fields (*114*). Orientation selectivity arises by inputs from the lateral

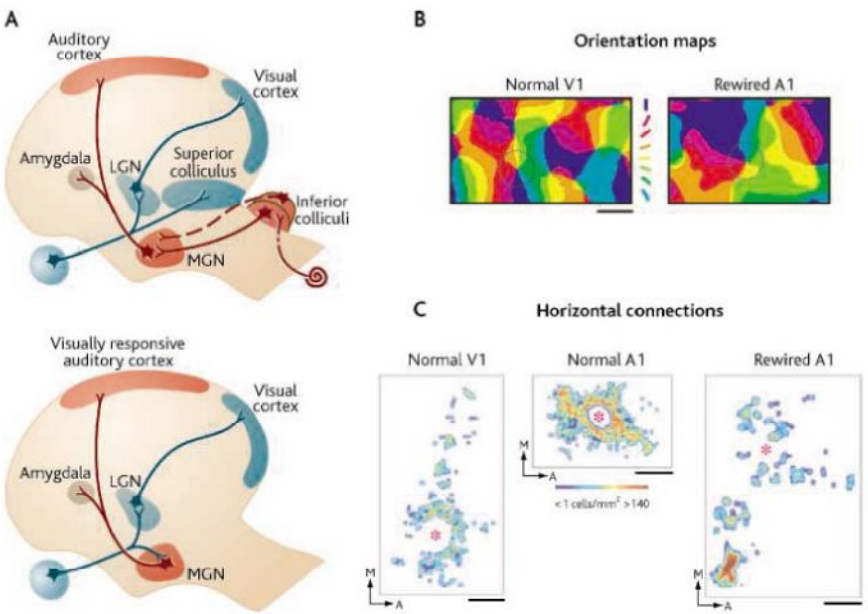

Fig. 4. Induction of function in cortex by novel activity. (**A**) Visual and auditory pathways in normal ferrets (top) originate from the retina and cochlea, respectively. Eliminating inferior colliculus projections to the medial geniculate nucleus (MGN) in neonatal animals results in retinal fibers innervating the MGN (bottom). The MGN still projects to the auditory cortex and amygdala but now relays visual information. Adapted from (*130*) and (*135*). (**B**) The orientation map in primary visual cortex (V1) of a normal ferret and in auditory cortex (A1) of a rewired ferret, revealed by optical imaging. The color of each pixel represents the stimulus orientation yielding the best response at that pixel (according to the key at right). As in normal V1, the map in rewired A1 contains pinwheels (within dotted circles) around which cells preferring different orientations are systematically represented (*133*). Scale bar, 0.5 mm. (**C**) Horizontal connections in ferret cortex. In V1, horizontal connections labeled with an injection of cholera toxin B (at starred site) in the superficial layers are patchy and link cells with similar orientation preference. In A1, horizontal connections spread along the isofrequency axis of cortex. In rewired A1, horizontal connections are patchy and resemble connections in V1. Colors indicate density of labeled cells according to the key at center. Scale bars, 0.5 mm. Adapted from (*133*).

geniculate nucleus that are aligned along the axis of orientation (*114*, *115*), although considerable evidence indicates that local intracortical excitation and balanced inhibition is critical for generating sharp orientation selectivity (*116*, *117*). Orientation-selective cells in at least the superficial layers of V1 are organized into an orientation map (*118*). Within the map, domains of cortical neurons that prefer a particular orientation are preferentially linked by long-range horizontal connections (*119*).

Orientation selectivity is present in V1 of monkeys at birth (*120*) and in cats and ferrets at eye-opening, although selectivity sharpens with visual experience (*121*, *122*). Visual deprivation impairs, but does not completely prevent, the development of orientation-selective responses (*121*, *122*). Although long-range horizontal connections are present in cats and ferrets just before eye-opening, the refinement of these connections depends on visual experience (*123*–*125*). Short-term monocular lid suture, after the orientation map has formed, leads to deterioration of the map driven by the closed eye; however, reopening the closed eye early in life restores the map (*126*, *127*). Thus, similar to ocular dominance columns, orientation selectivity and orientation maps in V1 may be set up by an early, intrinsic scaffold of connections that is later shaped by activity. Indeed, the orientation map forms synergistically with the maps of visual space and ocular dominance (*128*) and influences plasticity of orientation tuning in adult V1 (*129*).

Exposure of the cortex to novel sensory information provides complementary insights into the role of activity in development of orientation networks (*130*). Rearing kittens under conditions in which they view one particular orientation causes an overrepresentation of that orientation in V1 (*131*). Rewiring retinal inputs into the developing auditory thalamus causes auditory cortex to be driven by visual activity (Fig. 4A) (*132*). Primary auditory cortex in "rewired" ferrets develops visual orientation-selective cells and an orientation map (Fig. 4, B and C), with considerable reorganization of horizontal connections in a manner that supports the novel map (*133*). Yet, the connections retain vestiges of connections in normal auditory cortex, and the orientation map remains somewhat poorer than that in normal visual cortex (Fig. 4 B and C). Thus, visually driven activity interacts dynamically with earlier or ongoing developmental programs to shape network connections in the rewired cortex.

Finally, the novel projection from the retina to the auditory thalamus in rewired animals can profoundly affect behavior. Rewired ferrets trained to discriminate a visual from an auditory cue can perceive a visual cue as visual when the auditory cortex is activated by vision (*134*). Through direct activation of the amygdala via the auditory thalamus, rewired mice rapidly learn a visually cued conditioned fear response in a time comparable to an auditory cue (and much faster than a visual cue) in normal mice (*135*). Thus, the modality of inputs to the auditory thalamus can instruct the function of subsequent targets. Therefore, genetically determined brain pathways and cortical regions that are established during early development depend on their inputs for physiological and behavioral instruction.

Conclusions

The Protomap/Protocortex controversy no longer remains: It is clear that the parcellation of the cerebral cortex into discrete processing areas involves an interwoven cascade of developmental events including both intrinsic and extrinsic mechanisms. The field now has the intellectual foundation and tools that will enable it to elucidate more complex features of cortical development, such as the formation of higher order cortical areas and circuits (which are a robust feature of the primate brain) and the lateralization of cortical functions (*136*). Insights gained from such studies will undoubtedly facilitate understanding of the mechanisms underlying the evolution of neural systems that control cognition and emotion as well as the etiologies of disorders that derail them.

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REVIEW

Map Plasticity in Somatosensory Cortex

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Sensory maps in neocortex are adaptively altered to reflect recent experience and learning. In somatosensory cortex, distinct patterns of sensory use or disuse elicit multiple, functionally distinct forms of map plasticity. Diverse approaches—genetics, synaptic and in vivo physiology, optical imaging, and ultrastructural analysis—suggest a distributed model in which plasticity occurs at multiple sites in the cortical circuit with multiple cellular/synaptic mechanisms and multiple likely learning rules for plasticity. This view contrasts with the classical model in which the map plasticity reflects a single Hebbian process acting at a small set of cortical synapses.

A fundamental feature of neural circuits is the capacity for plasticity in response to experience or learning. A classic system for studying plasticity is primary somatosensory (S1) cortex. Somatosensory maps in S1 are highly plastic, both during development (*1*, *2*) and in adult animals (*3*). Plasticity occurs in response to peripheral lesions, passive sensory experience, and training on sensory tasks and is correlated with sensory perceptual learning. The underlying cellular mechanisms for map plasticity and its consequences for cortical processing are highly relevant to development, learning, and recovery of function after injury.

Rodent S1 cortex has emerged as a key model system in the analysis of the forms and mechanisms of map plasticity because of several experimental advantages. First, rodent S1 contains an orderly map of the large facial whiskers, which act as active tactile detectors, and large-scale map plasticity can be simply induced by trimming or plucking subsets of whiskers. Second, layer 4 (L4) of S1 contains an anatomical map of cell clusters, called "barrels," that is isomorphic to the arrangement of whiskers on the snout (*4*). Barrels can be visualized in brain slices, allowing cells and circuits at specific locations in the whisker map to be investigated in detail in vitro (*5*). Third, the superficial location of S1 allows live, optical imaging of neuronal function and structure, as well as whole-cell recording to study subthreshold events in vivo. Finally, molecular mechanisms of plasticity can be tackled using mouse genetics (*6*). Research on barrel cortex plasticity is particularly fascinating because of the wide range of techniques (*7*)—genetics, cell biology, in vitro and in vivo physiology, optical imaging—that are applied in the field. Here, we present an emerging consensus from these techniques that map plasticity is a distributed, multifaceted

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process with multiple synaptic and cellular mechanisms.

S1 Circuits and the Normal Whisker Map

A functional map of whisker receptive fields exists in S1, constructed by highly specific microcircuits whose anatomy and synaptic physiology are known in unprecedented detail. In the classical thalamocortical pathway (Fig. 1), afferents from the thalamic ventral posterior medial nucleus (VPM) innervate the L4 barrel corresponding to each whisker. Excitatory neurons in each barrel then project to L2/3 neurons in the same radial column. This feedforward intracolumnar pathway drives strong responses to each column's "principal whisker." Spread of excitation along cross-columnar pathways, together with broad tuning of thalamic inputs, confers weaker responses to neighboring, surround whiskers. Multiple types of inhibitory interneurons refine receptive fields and temporal response features. In a second afferent pathway, the septa between barrels in L4 receive less focused, multiwhisker input from the thalamic posterior medial nucleus. The result is a map in which each whisker activates a cortical region slightly larger than the anatomical column defined by its barrel (*8*) (Fig. 1). Synaptic connections between many identified cell classes have been quantitatively characterized (*8*–*11*), which suggests that, within the foreseeable future, it will be possible to identify cell type–specific synaptic weight and connectivity changes underlying S1 map plasticity.

Development of the Barrel Map

Both genes and neural activity instruct development of S1 maps. Signaling molecules partition the early cortex into specific subdivisions (*12*), as demonstrated by the duplication of the barrel field after electroporation of the signaling molecule fibroblast growth factor 8 (FGF-8) (*13*). Thus, thalamic afferents recognize gradients of signaling molecules in an early and intrinsically specified somatosensory cortex (*13*), rather than instructing a tabula rasa–like cortical sheet. Barrel formation within the prespecified S1 is instructed by peripheral afferents (*1*) and involves multiple, activity-dependent processes. These processes have begun to be revealed by genetic approaches [for review, see (*14*)].

Forms of Map Plasticity in S1

Sensory manipulations alter S1 maps. Multiple, distinct forms of map plasticity are seen depending on the pattern of sensory input, behavioral context, and age. Two basic principles generally hold. First, whisker manipulations early in life (the first postnatal week) cause rapid map plasticity in L4, consistent with plasticity at thalamocortical synapses (*6*). In older animals, however, plasticity tends to occur first in L2/3 and L5, and only later or not at all in L4 (*15*–*18*), although exceptions can occur (*19*). This suggests that L4 thalamocortical synapses exhibit an early critical period for rapid plasticity, whereas intracortical synapses in other layers remain highly plastic and are the primary places where rapid plasticity occurs throughout life. Second, changes in whisker use or activity drive plasticity of the whisker receptive field map, but only lesions of primary afferents disrupt anatomical patterning of barrels and only in neonates (*1*). Thus, use-dependent and lesion-dependent plasticity are mechanistically distinct (*6*, *14*).

Hebbian plasticity in response to preferential whisker use or training. In the classical form of map plasticity as originally defined in visual cortex, differential use of two sensory inputs causes the representation of the overused input to expand and that of the underused input to shrink. This is termed Hebbian plasticity because it follows Hebbian synaptic plasticity rules (*20*) and is commonly hypothesized to increase the cortical processing capacity of behaviorally relevant inputs. Hebbian plasticity occurs in nonwhisker S1 in response to overuse or preferential training of small regions of the hand or paw (*3*) and in whisker S1 in response to trimming or removing a subset of whiskers, which increases the behavioral salience of spared whiskers (*6*, *15*, *16*, *21*), or by appetitive or aversive conditioning of specific whiskers (*22*). The result is that spared or trained inputs expand in the S1 map, and deprived or untrained inputs shrink (Fig. 2).

Components of Hebbian plasticity. Hebbian plasticity in S1 has two

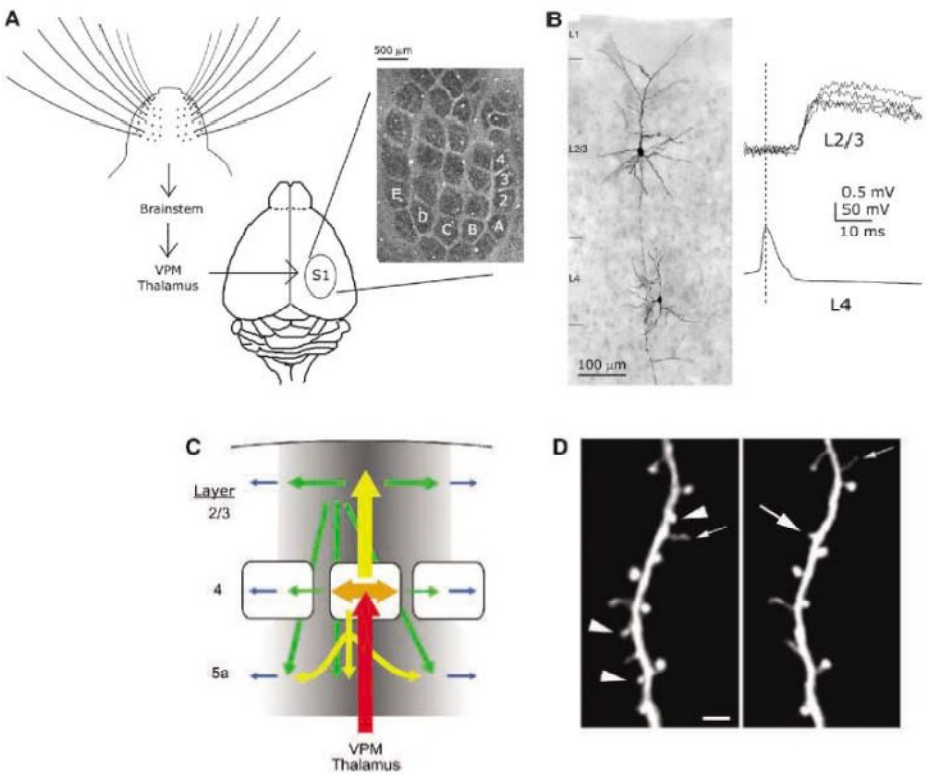

Fig. 1. Functional circuits in S1 cortex. (**A**) Pathway from whiskers to S1. (Inset) Cytochrome oxidase–stained barrels in layer 4 of S1. Letters and numbers indicate whisker rows and arcs. (**B**) Schematic flow of excitation evoked by single whisker deflection. Only the VPM input to cortex is considered. Order of events progresses from red to yellow to green to purple. Response strength is denoted by arrow thickness (*6*). Gray, cortical area with strong or moderate spiking responses to the whisker. (**C**) Example of characterization of synaptic physiology in S1, for a unitary connection from an L4 spiny stellate cell to a simultaneously recorded L2/3 pyramidal cell (*11*). Traces show excitatory postsynaptic potentials (EPSPs) (top right) evoked by single action potentials the L4 cell (bottom right). (**D**) Dynamic dendritic spines revealed by long-term in vivo two-photon imaging in S1 of an adolescent (1-month-old) rat [from (*82*)]. (Left) Apical dendritic segment from a layer 5 pyramidal cell. (Right) The same dendritic segment 2 weeks later. Arrowheads and arrows show spine elimination and formation. Thin arrows mark dynamic filopodia. Scale bar, 2 μm.

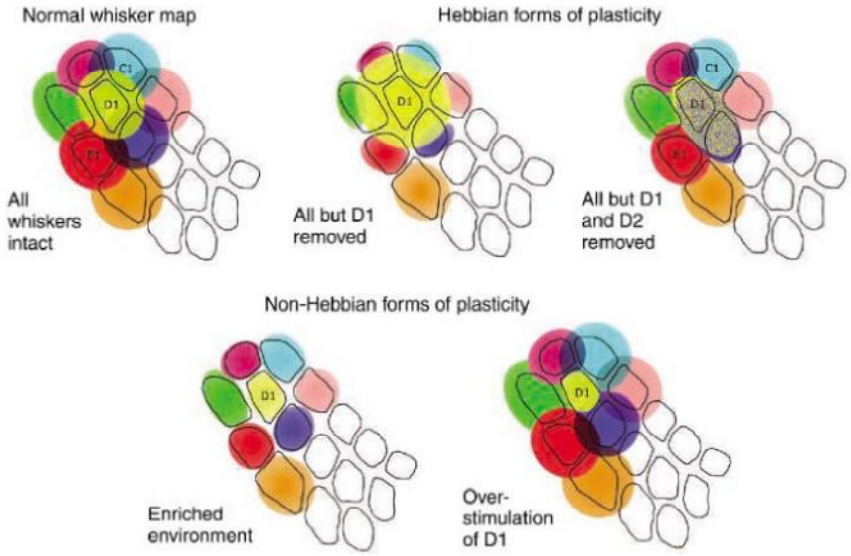

Fig. 2. Forms of whisker map plasticity in S1 cortex. In these schematized functional whisker maps in L2/3 of S1, colored regions represent cortical areas responding to different whiskers, with color saturation coding response strength. In normal rats, each whisker activates a cortical area slightly larger than the cortical column defined by its L4 barrel (barrels outlines are shown in black) (*8*). Removing all but the D1 whisker in adolescent rats causes Hebbian expansion of the spared, D1 whisker and weakening of deprived, surrounding whiskers within the map (*6*). Removing all but D1 and D2 whiskers causes D1 and D2 to merge within the map but not to expand into deprived columns (*16*). In two non-Hebbian forms of map plasticity, exposure to a novel, naturalistic environment sharpens the whisker map and weakens whisker responses (*18*), and overstimulation of a single whisker causes that whisker to shrink within the map.

separable components, which implies two mechanistically distinct processes for plasticity. In the first component, whisker deprivation selectively weakens neural responses to deprived whiskers, causing deprived whisker representations to shrink (*15*, *21*, *23*). Weakening is an active process that requires cortical spiking and is partly driven by competition from spared neighboring whiskers. One cellular basis for this component of plasticity is deprivation-induced weakening of the L4 to L2/3 (L4-L2/3) excitatory projection (*15*), which has been directly observed in S1 slices from whisker-deprived rats (*24*, *25*).

In a second, developmentally and genetically independent (*6*, *26*) component of Hebbian plasticity, responses to spared whiskers become enhanced (*15*, *21*, *27*, *28*). When isolated whiskers are spared, enhancement of spared whisker responses occurs in surrounding deprived columns, causing the spared whisker representation to expand in the S1 map (*15*, *21*, *27*). When multiple neighboring whiskers are spared, enhancement occurs instead in neighboring spared columns, which causes the representations of individual spared whiskers to merge or overlap (*16*) (Fig. 2). The latter case exemplifies classical Hebbian strengthening of coactive inputs onto common targets ("Neurons that fire together wire together"), which is a robust feature of map plasticity (*29*). Both cases may reflect enhanced transmission on excitatory, cross-columnar pathways into deprived or spared columns (*6*, *30*).

Deprivation of all whiskers degrades map topography. Trimming all whiskers during a narrow critical period at the peak of L2/3 synaptic development causes L2/3 neurons to adopt broad, unfocused receptive fields and a disordered whisker map, while the L4 map remains normal (*17*). This degraded map topography reflects increased cross-columnar (relative to within-column) input to L2/3 neurons (*31*) and disruption of normal barrel-septal segregation within the L4-L2/3 projection such that L2/3 neurons receive abnormally strong input from L4 septa, which have broad, poorly ordered fields (*25*). This suggests that developing barrel and septal inputs may compete for L2/3 targets, with experience driving normal segregation of these pathways.

Decreased representation of overstimulated whiskers. Several forms of plasticity cannot be explained by Hebbian or activity-based competitive mechanisms. Sustained, 24-hour passive stimulation of a whisker causes the representation of the activated whisker to weaken and to shrink in adult S1 (*32*). This plasticity occurs in L4 and is correlated with an increase in number and density of GABAergic synapses onto L4 spines (*19*). This effect may represent a homeostatic mechanism to normalize firing rates and/or a habituation process to reduce responses to repeated, behaviorally insignificant input.

Regulation of map precision and signs of plasticity by sensory enrichment. Transferring adult rats from familiar home cages into complex natural environments causes another non-Hebbian form of plasticity in which whisker representations contract in L2/3, thus sharpening the whisker map (Fig. 2). L4 receptive fields are unaffected (*18*). Similar map sharpening occurs rapidly during acute arousal and exploration (*33*). One possible mechanism is that environmental novelty upregulates arousal-related modulators, which are known to act in cortex to shrink whisker representations (*33*). Exposure to a novel environment for only a few minutes per week, which is not enough to sharpen the whisker map, also has the profound and unexplained effect of reversing the sign of Hebbian plasticity: When all but one whisker are removed, the representation of the spared whisker shrinks, rather than expands (*34*). The existence of these functionally distinct forms of plasticity indicates that multiple cellular plasticity mechanisms and learning rules act in S1, beyond canonical Hebbian plasticity mechanisms.

Physiological Mechanisms of Plasticity

Substantial progress has been made in S1 in identifying the underlying cellular mechanisms for Hebbian and other forms of map plasticity. In classical models, rapid components of Hebbian plasticity reflect long-term potentiation (LTP) and depression (LTD) at cortical synapses; slower components reflect anatomical rearrangement of cortical microcircuits (*3*). Competition between inputs, which is often associated with Hebbian map plasticity, is not directly predicted from Hebbian synaptic plasticity rules and may require an additional cellular mechanism (*35*, *36*). S1 experiments support certain aspects of this model (e.g., involvement of LTP and LTD in Hebbian plasticity), but refute others (e.g., that anatomical plasticity must be slow to occur). Mechanisms for non-Hebbian forms of plasticity are also emerging (*19*, *36*).

LTP and LTD. Many S1 synapses exhibit *N*-methyl-D-aspartate (NMDA) receptor-dependent LTP and LTD, and the capacity for LTP and LTD correlates with critical periods for map plasticity in each layer (*37*). Pharmacological blockade or transgenic deletion of cortical NMDA receptors impairs barrel development (*14*) and refinement and plasticity of receptive fields (*38*–*40*). During Hebbian map plasticity, the enhancement of spared whisker responses is abolished or impaired in mice lacking functional α-CaMKII (calcium/calmodulin-dependent protein kinase II, type α) or α/δ CREB [cyclic adenosine monophosphate (cAMP) response element binding protein], or expressing autophosphorylation-incompetent α-CaMKII, all of which are required for cortical LTP (*6*). Thus, LTP [or CaMKII/CREB-

dependent structural rearrangements related to LTP (*41*)] is a likely substrate for this component of plasticity. The synaptic locus for LTP may be excitatory pathways from spared to neighboring columns, potentiation of which would expand the spared whisker representation.

LTD, or an LTD-like synaptic weakening, appears to be a major substrate for the shrinkage of deprived whisker representations during Hebbian map plasticity. Weakening of the excitatory L4-L2/3 projection has been detected physiologically after partial whisker deprivation, in ex vivo S1 slices prepared from whisker-deprived rats (*24*, *25*). This weakening occurs without loss of L4 neurons, axonal boutons, or changes in postsynaptic excitability (*24*, *42*, *43*). Instead, deprivation-induced weakening occludes LTD and shares apparent presynaptic expression with LTD, which suggests that it represents LTD induced in vivo (*24*, *44*). Whether this reflects physiological weakening of preexisting synapses, synapse elimination, or both, is unknown. Conversely, normal whisker use drives measurable LTP at L4-L2/3 synapses (*45*), which indicates that L4-L2/3 synapses are a site of bidirectional, experience-dependent plasticity in S1.

Other physiological mechanisms of plasticity. LTP and LTD at excitatory synapses are not the only mechanisms for cortical plasticity. Short-term synaptic dynamics are altered by sensory experience (*5*). Inhibitory circuits are also altered: Levels of γ-aminobutyric acid (GABA), GABA type A α1 receptors, and the GABA-synthesizing enzyme GAD67 (glutamic acid decarboxylase) are regulated by sensory deprivation and sensory learning, and the number and density of GABA synapses in L4 are decreased by whisker deprivation and increased by passive stimulation (*46*). In addition, an apparently large number of barrel cortex neurons exhibit very low firing rates (*47*); recruitment of these silent neurons into the active neuronal population could be an important plasticity mechanism (*48*). The diversity of plasticity mechanisms identified in the few existing studies suggests that additional mechanisms remain to be discovered.

Learning Rules for Plasticity

The quantitative relationship between pre- and postsynaptic activity parameters and resulting synaptic plasticity is termed the synaptic learning rule. A central dogma is that experience drives plasticity via local, sensory-evoked activity patterns that engage these learning rules (*20*). A major focus of research is to determine the relevant learning rules and network activity patterns that drive plasticity in vivo. Best studied are learning rules for LTP and LTD, which include rate-dependent rules in which high- and low-frequency presynaptic firing, respectively, drive LTP and LTD, and spike timing-dependent plasticity (STDP) rules in which changes in millisecond-scale timing of pre- and postsynaptic spikes drive LTP and LTD largely independent of firing rate (*49*).

STDP and Hebbian synaptic plasticity. The relevant learning rule for plasticity has been studied for deprivation-induced LTD at L4-L2/3 synapses, which contributes to Hebbian weakening of deprived whisker representations. L4-L2/3 synapses exhibit both rate-dependent plasticity and STDP in vitro (*24*, *50*). In STDP at this synapse, LTP is induced when the L4 cell fires 0 to 15 ms before L2/3 cells, and LTD is induced when firing order is reversed, for spiking delays of 0 to 50 ms (*50*). STDP learning rules biased toward LTD are common for cortical pyramidal cells, inherently drive Hebbian plasticity, and predict LTD in response to either reliable, postleading-prefiring or to uncorrelated spiking (*50*). In vivo firing patterns suggest that STDP is the relevant learning rule by which whisker deprivation drives LTD: When all whiskers are deflected together to mimic normal whisking in anesthetized animals, L4 neurons spike reliably before L2/3 neurons. However, when all whiskers except the principal whisker are deflected to mimic acute whisker deprivation, L4-L2/3 firing decorrelates and mean firing order reverses. These spike timing changes are quantitatively appropriate to predict spike timing–dependent LTD (*51*). In contrast, acute deprivation changes mean firing rate only modestly and insufficiently to predict rate-dependent LTD (*51*). Thus, spike timing, not spike rate, may be the key parameter that drives synaptic weakening during Hebbian plasticity in S1.

Neuromodulation. Hebbian plasticity is enhanced by behavioral relevance and attention, particularly in adults. Attentional gating of plasticity may be provided by neuromodulators such as acetylcholine (ACh) released in cortex by basal forebrain inputs. Map plasticity in S1 and other areas requires ACh, and pairing of whisker stimuli with ACh application drives receptive field plasticity (*52*). This suggests that ACh and other modulators may fundamentally gate or modify Hebbian learning rules during appropriate behavioral contexts.

Competition between inputs. Competition between spared and deprived inputs drives key aspects of S1 plasticity (*6*), but the biological mechanisms and learning rules for competition are almost entirely unknown. In one proposed mechanism, Hebbian learning rules themselves change as a function of postsynaptic activity, so that depriving one set of inputs increases the likelihood that remaining, spared inputs will strengthen (*35*). STDP provides an alternative explanation for competition, because multiple inputs actively compete in STDP models for control of spike timing. Competition could also be implemented by non-Hebbian, homeostatic forms of plasticity (*36*), or by anatomical competition for synaptic space by dynamic axons and dendrites. However, the actual mechanisms of competition in vivo remain unknown.

Structural Changes

In the last few years, classical structural analysis of cortical circuits based on static, postmortem tissue has been revolutionized by the study of dynamic, living neurons expressing fluorescent proteins (*53*) and visualized in vivo by two-photon imaging (*54*, *55*). This technique has revealed that cortical circuits are structurally highly dynamic and are regulated by sensory experience (Fig. 1D; Fig. 3). Accordingly, even rapid components of cortical map plasticity could be mediated, in part, by structural changes in cortical microcircuits (*56*), and physiological changes in synapse strength may be closely linked to structural plasticity (*57*–*59*).

Dendritic branch dynamics. Early in life, dendritic branches are highly dynamic, and dendritic architecture is altered in response to whisker trimming (*60*), environmental enrichment (*61*), and peripheral lesion (*1*). In adults, basic dendritic branch structure in S1 is highly stable over weeks of normal sensory experience (*62*, *63*), and branching is unaffected by whisker trimming or plucking (*62*), although older studies suggest that complex environments can increase dendritic complexity (*64*). Peripheral lesions continue to drive robust dendritic branch plasticity in adults (*65*). In line with in vitro evidence (*66*), one might speculate that neurotrophic factors—or the lack thereof—trigger dendritic remodeling in response to lesions. Thus, structural changes in dendritic branches may contribute to developmental and lesion-induced plasticity but are unlikely to contribute to experience-dependent plasticity in mature animals.

Axonal dynamics. Cortical axonal trees are more difficult to visualize, and consequently, we have only limited information about cortical axonal dynamics in vivo. In visual cortex, there is massive, experience-dependent axonal remodeling during development (*67*), but it is not clear to what extent such axonal remodeling occurs in barrel cortex. Initial outgrowth of L4 axons into L2/3 during barrel cortex development is largely topographically specific (*68*) and is not affected by whisker plucking (*42*). While alterations of afferent input can alter axonal fields in adult visual cortex (*69*), the stability of the large-scale organization of the axonal network in the adult barrel cortex remains to be investigated (*56*).

Spine dynamics. Dendritic spines (*70*) are important biochemical compartments in cortical processing, and spine motility and turnover have been the focus of numerous in vivo imaging studies (*62*, *63*, *71*, *72*). These studies indicate that spines can be highly dynamic structures, with dynamics regulated by senso-

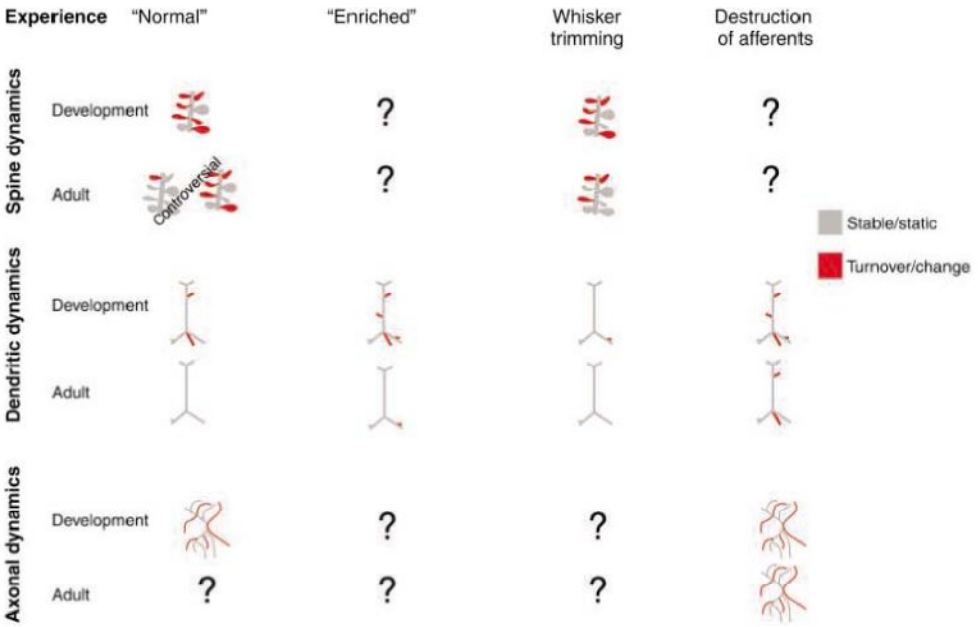

Fig. 3. Experience induced structural changes in S1 cortex. Schematic representation of experience- and deprivation-induced alterations in barrel cortex circuitry. Spine data refer to chronic in vivo imaging experiments (*62*, *63*, *71*, *72*). Dendritic data were collected in chronic in vivo imaging experiments (*11*, *64*) or in conventional anatomical experiments (*1*, *18*, *60*, *61*, *64*). The effects of sensory enrichment include data from non-S1, as well as S1, barrel cortex.

ry experience. Although there are some quantitative disagreements, these studies agree on a number of basic facts: (i) Spines are dynamically added and eliminated in vivo over a time course of hours (spine turnover). (ii) Turnover decreases with age. (iii) Spines are heterogeneous and differ in their turnover rates. (iv) Thick bulbous spines have lower turnover rates than thin spines. (v) There is a net loss of spines in late postnatal development.

In the developing brain there is massive motility of filopodia and high turnover rates of spines, and spine dynamics are regulated by experience (*55*, *62*). Using in vivo two-photon imaging and subsequent electron microscopic reconstruction of imaged spines, it was shown that many dendritic protrusions in S1 carry synapses, but that synapses are probably absent from sites of recently retracted spines, which suggests that spine sprouting and retraction are associated with synapse formation and elimination (*62*). Conclusive proof of this important point may be obtained in the future by imaging markers for synaptic structures [e.g., AMPA receptors (*73*)]. Such approaches, which can also be applied to presynaptic structures, will also take the field from imaging what we can see best (anatomical protrusions on dendrites) toward what we are interested in most (functional synaptic connections). In the adult brain, spines are more stable, but details remain controversial. Authors agree that large thick spines are more stable than thin spines (*63*, *71*, *72*), but disagree whether 75% (*71*) or 95% (*63*, *72*) of spines are stable over weeks in the adult brain. Complicating these findings is disagreement on the classification of spines versus other dendritic protrusions. Thus, it is unclear if what one group (*63*, *72*) considers a filopodium [a long, thin protrusion lacking a bulbous head (*72*)], is considered a thin spine by another group (*62*, *71*, *73*). Post hoc ultrastructural analysis by electron microscopy (EM) will help resolve this issue. Further scrutiny of experimental details like brain exposure, pharmacological treatments, animal strain, and housing conditions is required to compare spine turnover across groups and to determine its role in cortical plasticity.

Several important future directions are obvious in the analysis of structural plasticity of barrel cortex. The first is to devise strategies to independently analyze structural dynamics of identified cell types within specific intracortical circuits. A second issue is the origin of wiring specificity. Pairs of neighboring excitatory barrel cortex neurons are either unconnected or share four or five synaptic terminals (*9*, *10*, *74*). This scenario is dramatically different from what is expected for a probabilistic connectivity, which—based on axonal and dendritic geometries—predicts neighboring cortical neurons to be connected usually by one terminal, rarely by two, and almost never by three terminals or more (*75*). The origin of such precise wiring, whether activity-dependent processes and/or genetic cues, is entirely unclear.

A third major issue is to understand how structural plasticity is related to functional changes in synaptic efficacy like LTP and LTD. In vitro, late phases of LTP and LTD are correlated with synapse and spine formation and elimination (*57*, *58*). Thus, activity may rapidly regulate synaptic efficacy by LTP and LTD, which in turn may modulate structural dynamics and lead to long-term effects on morphology of axons and/or dendrites (*59*). When examined, most vertebrate studies in vivo report parallel changes in synaptic structure and function, but the alternate possibility that structural and functional plasticity are controlled independently via dissociable signaling pathways, as reported in invertebrates (*76*), cannot be ruled out at present.

Outlook and Summary

Ramón y Cajal once pointed out that the cortex is a very difficult matter, a tissue of endless complications, where any kind of simplistic approach is bound to fail (*77*). A strength of the work on S1 plasticity has been to avoid such simplification. S1 map plasticity is not a unitary phenomenon but has many distinct forms with multiple components, cellular mechanisms, and sites of plasticity. Similar complexity is likely to exist in other cortical areas.

Where do we go from here? Some of the most promising approaches lie in the combination of novel genetic, optical, and physiological techniques. Recent improvements in gene transfer methods allow sparse transfection and genetic alteration of cells in an otherwise intact brain (*78*, *79*). Transfected cells can then be electrophysiologically analyzed by two-photon targeted patch recordings in vivo (*80*) in order to detect effects on development and plasticity of sensory responses. The tremendous spatiotemporal specificity of such manipulations will help determine how genes or single-cell activity patterns contribute to systems-level properties like plasticity.

A second challenge is to identify additional synaptic learning rules that drive plasticity in vivo. Here, one obvious approach is to utilize recent advances in multisite recording techniques to characterize the network activity patterns that occur naturally in vivo to drive map plasticity. A third challenge is to develop the computational tools and theoretical framework necessary to understand how the multiple discrete mechanisms and sites of plasticity, including both functional and structural changes, work together in cortical circuits to produce overall map plasticity. Finally, future research must address the behavioral and perceptual consequences of barrel cor-

tex plasticity, which are—with few exceptions (*81*) poorly understood. Although complex, a mechanistic, cellular-level explanation of S1 map plasticity appears increasingly tractable and would constitute a major step toward understanding cortical information storage.

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VIEWPOINT

Language Acquisition and Brain Development

Kuniyoshi L. Sakai

Language acquisition is one of the most fundamental human traits, and it is obviously the brain that undergoes the developmental changes. During the years of language acquisition, the brain not only stores linguistic information but also adapts to the grammatical regularities of language. Recent advances in functional neuroimaging have substantially contributed to systems-level analyses of brain development. In this Viewpoint, I review the current understanding of how the "final state" of language acquisition is represented in the mature brain and summarize new findings on cortical plasticity for second language acquisition, focusing particularly on the function of the grammar center.

A child acquires any natural languages within a few years, without the aid of analytical thinking and without explicit "grammar" instruction as usually taught in school. The origin of grammatical rules should thus be ascribed to an innate system in the human brain (*1*). The knowledge of and competence for human language is acquired through various means and modality types. Linguists regard speaking, signing, and language comprehension as primary faculties of language, i.e., innate or inherent and biologically determined, whereas they regard reading and writing as secondary abilities. Indeed, the native or first language (L1) is acquired during the first years of life through such primary faculties while children are rapidly expanding their linguistic knowledge (*2*). In contrast, reading and writing are learned with much conscious

effort and repetition, usually at school. This ability may be influenced by cultural rather than biological factors. However, the existence of developmental dyslexics indicates that reading ability requires specific neural mechanisms (*3*), and a link between poor reading and impaired auditory resolution has been suggested (*4*). It is therefore crucial to understand how distinct linguistic faculties develop in the brain throughout various ages. Figure 1 illustrates the typical development of L1 faculties. This correlates with a massive increase in brain volume during the first years. Speech in infants develops from babbling at around 6 to 8 months of age, to the one-word stage at 10 to 12 months, and then to the two-word stage around 2 years. Note that sign systems are spontaneously acquired by both deaf and hearing infants in a similar developmental course (*5*), starting from manual silent "babbling" (*6*). However, these obvious developmental changes refer to language output. Speech perception and even grammatical knowledge develops much earlier, within the first months after birth (*7*, *8*).

A clear contrast among linguistic factors exists between L1 and a second language (L2). The L2 ability does not seem to take any determined steps of development, and it shows enormous individual variation. Whether L2 relies on the same dedicated mechanism of L1 is thus a matter of debate (*9*). An L2 can be mastered at any time in life, though the L2 ability rarely becomes comparable to that of L1 if it is acquired beyond the hypothesized "sensitive period" from early infancy until puberty (~12 years old). The notion of a sensitive period for language acquisition comes from the loss of flexibility for cerebral reorganization due to acquired aphasia after puberty (*10*). The concept of the sensitive period has been extended to L2 acquisition in that English proficiency declines after the age of 7 years when Chinese or Korean speakers move to the United States (*11*). This hypothesis has recently been challenged by an event-related brain potential (ERP) study. Adults who learned a miniature artificial language showed a similar ERP response to a syntactic anomaly as native speakers do (*12*). It may also be possible that different linguistic abilities are acquired in their own developmental courses and that the timing and duration of their sensitive periods differ. In this viewpoint, I will first clarify the fundamental linguistic factors and their possible representation in the mature brain as revealed by brain mapping techniques. The major linguistic factors are phonology and lexico-semantics at the word level and sentence comprehension and syntax at the sentence and discourse level, which certainly interact with each other (Fig. 2A). A critical question is whether these factors correspond to distinct regions of the brain. I will then focus on advances in functional imaging studies of L2 acquisition, indicating activation changes in particular regions of the brain during the course of language development.

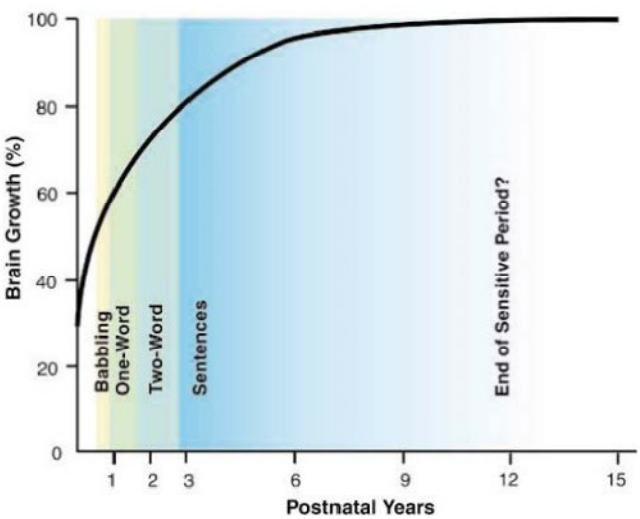

Fig. 1. Brain growth and first language (L1) acquisition. Human brain weight is presented as a function of age, where 100 in the ordinate corresponds to the mean adult value (*10*). Approximate times of milestones in normal speech development are also indicated.

Phonology and Lexico-Semantics

Recent functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) studies have indicated that auditory phonological processing is associated with activation in the posterior superior temporal gyrus (STG) [Brodmann's area (BA) 22], whereas lexico-semantic processing is typically associated with activation in the left extra-sylvian temporoparietal regions, including the angular gyrus and supramarginal gyrus (AG/SMG) (Fig. 2A) (*13*). However, studies on phonological versus lexico-semantics have reported many additional regions, including the inferior frontal regions, and phonological processing may have varied levels of abstraction within distinct subregions (*14*). We have shown that bilateral STG activation is more enhanced in phonological decision and voice-pitch comparison tasks than in syntactic and semantic decision tasks even when the same speech stimuli are used (*15*). On the other hand, activations in the left AG/SMG and frontal regions are less consistent among the lexico-semantic tasks tested by a number of functional imaging studies. Lexico-semantic tasks may involve various cognitive factors other than semantic processing, and thus different cortical regions might be recruited depending on the particular strategy used by the participants.

Sentence Comprehension

Sentences convey not only lexico-semantic information for each word but sentence meaning based on syntactic structures. Semantic processing at the sentence level differs from a simple summation of lexico-semantic processing for each word. For example, the meaning of "John thinks that David praises his son" clearly differs from that of "John thinks that his son praises David," although the lexical items involved in each of these sentences are identical. Therefore, the processing of syntactic structures plays a critical role in the selective integration of lexico-semantic information into sentence meaning. We proposed that the left inferior frontal gyrus (IFG) region extending from the triangular part (F3t or BA 45) to the orbital part (F3O or BA 47) is the putative region for the selection and integration of semantic information, which are separable from simple lexico-semantic processing (*16*) (Fig. 2B, green region). We directly compared cortical activations in tasks involving comprehension of sentences with those in lexical decision tasks and found discourse-level selective activation in the left F3t/F3O under both auditory and visual conditions. We also clarified that the functional connectivity between the left F3t/F3O and a region in the left precentral sulcus is significantly enhanced during the sentence task but not during the lexico-semantic task (*17*). In the neuroimaging field, there is a growing emphasis on structural and functional connectivity to clarify how distributed but interacting populations of neurons work in a coordinated fashion during language processing.

A recent fMRI study showed that the processing of American Sign Language (ASL) recruited the bilateral cortical areas of both deaf native signers and hearing native signers, whereas the processing of written English was left-lateralized (*18*). Note that for the deaf signers, ASL is the L1 and written English the L2. Another fMRI study reported

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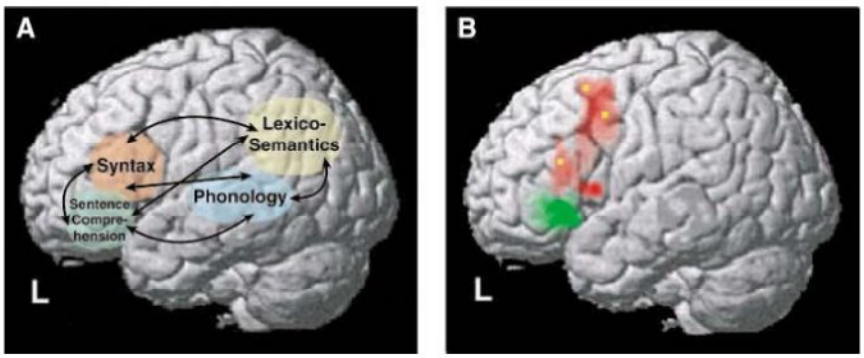


Fig. 2. (**A**) Possible network of fundamental linguistic functions in the brain. The exact correspondences between the left (L) brain regions and linguistic factors are still under study. (**B**) The grammar center and other left frontal regions critically involved in sentence processing. The green region (the left F3t/F3O) is selectively activated in the comprehension of sentences (*16*, *17*), whereas the red regions (the left lateral premotor cortex, the left dorsal IFG, and the left F3op/F3t) are specifically involved in syntactic processing (*15*, *26*) and can be regarded as the grammar center.

bilateral cortical activation for the processing of British Sign Language (BSL), but without evidence of enhanced right-hemisphere recruitments in sign language when compared with an audio-visual speech condition (*19*). It is, therefore, a considerable challenge to clarify "what's right and what's left." Because sign-language aphasia is due primarily to left-hemisphere lesions (*20*), it should be clarified whether comprehension of sentences is functionally lateralized in sign and speech. By using tasks involving comprehension of sentences and sentential nonword detection, we compared different groups and stimulus conditions (*21*). Under the sign condition with sentence stimuli in Japanese Sign Language (JSL), we tested two groups of participants: deaf signers of JSL and hearing bilinguals of JSL and Japanese. Under the speech condition, we tested hearing monolinguals of Japanese with auditory Japanese stimuli alone or with an audio-visual presentation of Japanese and JSL stimuli. Across all four conditions, there were consistently left-dominant activations involving frontal and temporo-parietal regions. Furthermore, irrespective of the modalities of sign and speech, activations selective to the comprehension of sentences were found primarily in the left regions, including the left F3t/F3O; only the left F3t/F3O showed no main effects of modality condition. These results indicate amodal commonality in the functional dominance of the left cortical regions for comprehension of sentences as well as the essential and universal role of the left F3t/F3O in processing linguistic information from both signed and spoken sentences.

Syntax: The Grammar Center

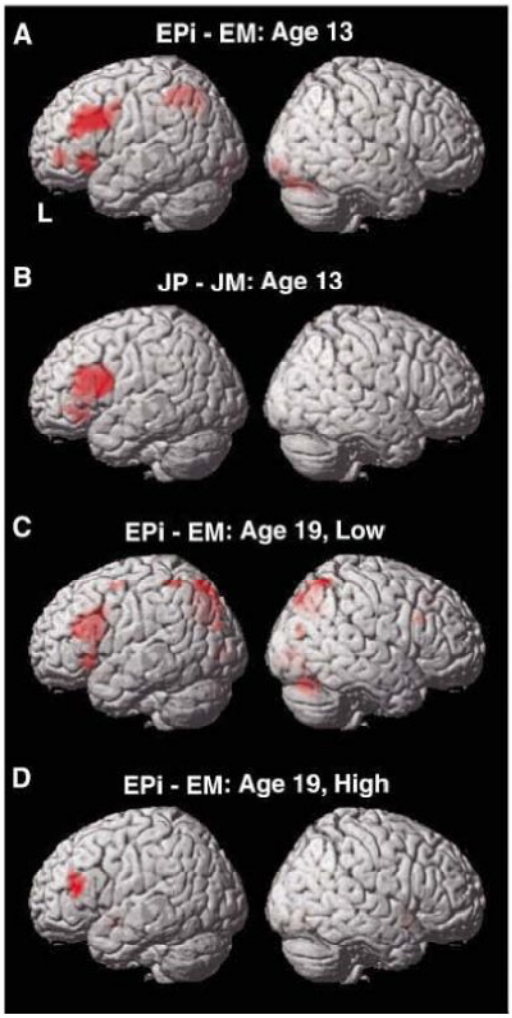
Although there has been much speculation concerning subdivisions for various aspects of sentence processing and consensus is still lacking, there is accumulating evidence that the opercular and triangular parts (F3op/F3t or BAs 44 and 45) of the left IFG and the left lateral premotor cortex (BAs 6, 8, and 9; mainly in BA 8) are selectively related to grammatical processing (*15*, *22*–*26*). The left lateral premotor cortex is located at the junction of the precentral sulcus and the inferior frontal sulcus and is just dorsal to the left F3op/F3t. I propose that these left frontal regions can be regarded as the "grammar center," reflecting the universal nature of grammatical processing. Is there a specialized (domain-special) neural system for grammatical processing that is separable from other domain-general cognitive systems? We examined cortical activation by directly comparing brain activations in syntactic decision tasks with those in verbal short-term memory tasks (*26*). The left dorsal IFG (a part of F3op/F3t) as well as the left lateral premotor cortex showed selective activation for syntactic decision tasks when they were directly compared with a verbal short-term memory task (Fig. 2B, red regions). The activation of these regions is related to processes of analyzing syntactic structures, and it cannot be explained either by task difficulty or by verbal short-term memory components. The human left frontal cortex is thus uniquely specialized in the syntactic processes of sentence comprehension, without any counterparts in other animals.

There is great controversy regarding the limits of noninvasive imaging techniques as a tool for human studies; for example, such correlation methods are insufficient to show causal relationships between cortical activations and linguistic functions. To establish a causal link between the grammar center and syntactic processing, we used transcranial magnetic stimulation (TMS) and a minimal-pair paradigm in which either a syntactic or semantic factor differed between stimulus pairs (*27*). Event-related paired TMS pulses over the left F3op/F3t selectively reduced reaction times in explicit syntactic decisions but not in explicit semantic decisions, suggesting the selective physiological effects of facilitation or priming. This effect was observed during syntactic decisions regarding both normal and anomalous sentences and when magnetic stimulation was administered to the left F3op/F3t at a specific time (150 ms from a verb stimulus). Even if the "normal" sentences were physically identical stimuli, TMS showed the differential effects on the normal sentences that paralleled the effects on anomalous sentences, depending on the types of explicit linguistic decisions being made. These results indicate that the left F3op/F3t plays an essential role as the grammar center of human sentence processing.

Functional Changes of the Grammar Center During L2 Acquisition

How can the function of the grammar center be modified during the acquisition of new languages? There are at least two major factors that may affect the cortical activation change: the proficiency level (PL) of L2 and the age of acquisition (AOA). It has been reported that L1 (AOA before about 6 years) and L2 (AOA after about 7 years) are represented differentially in cortical areas (*28*), whereas other studies have reported that they have common neural substrates during sentence comprehension tasks (*29*). An fMRI study supports the AOA effect on cortical activations, showing that the left IFG activation for grammatical processing in L2 is greater than that in L1 (*30*). However, another fMRI study claims that the degree of exposure to language affects the left IFG activation, even if the AOA is matched (*31*). It has also been pointed out that the left frontal and extrastriate regions are differentially modulated, either by age or task performance among children (aged 7 to 10) and adults (*32*). However, the age and PL effects on cortical activations are often confounded with the demands required in each language task and the resultant task performance, and it remains unknown whether these factors are actually separable from each other.

Given these uncertainties, we tried to clarify the relative contributions of age, PL, language task demands, and task performance to modulating activations in the left IFG. We examined whether learning of English past-tense verbs as L2 knowledge altered the brain activations of 13-year-old students (native Japanese speakers) studying English for the first time (*33*). We targeted twins as participants (six monozygotic and one dizygotic twin pairs), because it is intriguing to ask whether the shared factors of twins actually influence their language abil-



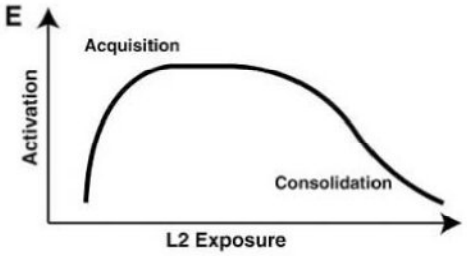


Fig. 3. Functional changes of the grammar center during second language (L2) acquisition. (**A**) Past-tense task-selective activation in L2 (EPi, the English past-tense task with irregular verbs) after classroom training for participants age 13 years (*33*). (**B**) Past-tense task-selective activation in L1 (JP, the Japanese past-tense task) for participants age 13 years. (**C**) Past-tense task-selective activation in L2 (EPi) for the lower PL subgroup of participants age 19 years (*34*). (**D**) Past-tense task-selective activation in L2 (EPi) for the higher PL subgroup of participants age 19 years. (**E**) Possible activation changes in the brain during L2 acquisition and consolidation.

ities and neural substrates. For 2 months, the students participated in intensive training in English verbs as part of their standard classroom education. The twins completed two sets of fMRI sessions, one before (day 1) and one after (day 2) training. When an English past-tense (EP) task was contrasted with an English verb-matching (EM) task for day 2, activations were found primarily in the left IFG (Fig. 3A); these activations had been absent in the same contrast for day 1. The contrast between Japanese past-tense (JP) and Japanese verb-matching (JM) tasks resulted in the same left IFG activation (Fig. 3B), which is in agreement with the universal nature of grammatical processing. These results suggest that cortical plasticity for L2 acquisition is guided toward L1 specialization of the left IFG, at least at the age of 13, despite notable differences between L1 and L2 in the students' linguistic knowledge and in their performance in making past-tense forms. The activation increases of the left dorsal IFG across days 1 and 2 showed a highly significant correlation within each pair of twins. This suggests that the functional changes specifically observed in the left IFG were susceptible to shared genetic and environmental factors for each twin in a surprisingly predictive manner. The activation increases in the left IFG predicted the extent to which the individual participants improved their knowledge of the past tense. In a subsequent fMRI study, we tested participants aged 19 who had studied English for 6 years, thereby comparing the cortical activations involved in the above-mentioned EP and EM tasks (*34*). The activation in the left dorsal IFG was lower, corresponding to a higher PL (Fig. 3, C and D), suggesting that the PL plays a major role in the activation of this region. On the other hand, the left F3t/F3O activation in Japanese (L1) of participants aged 13 was significantly greater than that for those aged 19, despite the matched performances in L1. We conclude that the grammar center subserves specific linguistic functions that are critically required when mastering any language.

Combining these task-selective activation changes, left dorsal IFG activation increases with PL improvements at the early stages of L2 acquisition and becomes lower when a higher proficiency in L2 is attained. These results may reflect a more general law of activation changes during language development. Cortical activations increase initially at the onset of acquisition, followed by the maintenance of the activations and then a fall in activations during consolidation of linguistic competence (Fig. 3E). On the other hand, the developmental changes in regional cerebral blood flow and cerebral metabolic rates are known to manifest initially as an increase and later, after about the age of 9, as a decrease (*35*). Because such metabolic differences between children and adults might affect the acquisition, analysis, and interpretation of fMRI data in group analyses, an appropriate task control is necessary to compensate for the global physiological changes in the brain. Moreover, if the general law stated above is applicable, a brain region may show higher, lower, or comparable activation, depending on which developmental stages are compared.

Outlook

Noninvasive imaging techniques have already been applied to study the "initial state" of brain activations reflecting speech perception in infants (*36*, *37*). In the future, participants at various developmental stages will be systematically tested by functional imaging studies with language and/or general cognitive tasks. Regional cerebral volume and tissue concentration differences have also been characterized by voxel-based morphometry, and this technique may elucidate structural development of the brain in a larger population, extending the study of adult human brains (*38*). Indeed, twin studies have contributed to reveal genetic factors for brain structure, more significantly those influencing language areas in the left hemisphere (*39*). Longitudinal studies of both structure and function of brains may further reveal their developmental tendencies in general, as well as individual differences. Moreover, the observation of functional changes during recovery from neurological conditions, such as dyslexia and aphasia, will help facilitate remediation and rehabilitation in both children and adults. As to the normal development of the brain, further research is still

necessary to determine whether the left IFG activation depends on exposure to L1 and L2 at a particular stage, thus clarifying the existence of a sensitive period. Future studies will investigate how individual subregions of the left frontal cortex, as well as other cortical regions, work in concert and subserve human-unique language acquisition. This promising approach to evaluating developmental changes in terms of not only indirect behavioral changes but direct brain changes is taking a first step toward a new era in the systems neuroscience of human language.

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VIEWPOINT

Sex Differences in the Brain: Implications for Explaining Autism

Simon Baron-Cohen,* Rebecca C. Knickmeyer, Matthew K. Belmonte

Empathizing is the capacity to predict and to respond to the behavior of agents (usually people) by inferring their mental states and responding to these with an appropriate emotion. Systemizing is the capacity to predict and to respond to the behavior of nonagentive deterministic systems by analyzing input-operation-output relations and inferring the rules that govern such systems. At a population level, females are stronger empathizers and males are stronger systemizers. The "extreme male brain" theory posits that autism represents an extreme of the male pattern (impaired empathizing and enhanced systemizing). Here we suggest that specific aspects of autistic neuroanatomy may also be extremes of typical male neuroanatomy.

Leaving aside political correctness, there is compelling evidence for sexual dimorphism in the brain, cognition, and behavior (*1*). In this Viewpoint, we review the evidence at all three levels. Classic autism and Asperger syndrome (AS) are the two clearest subgroups on the autistic spectrum of conditions, and both affect males more often than females. We conjecture that understanding sex differences in the general population has implications for understanding the causes of autism-spectrum conditions.

The E-S Theory of Psychological Sex Differences

Although males and females do not differ in general intelligence, specific cognitive tasks reveal sex differences. Differences favoring males are seen on the mental rotation test (*2*), spatial navigation including map reading (*3*), targeting (*4*), and the embedded figures test (*5*), although there are conflicting studies regarding the latter (*6*). Males are also more likely to play with mechanical toys as children (*7*), and as adults, they score higher on engineering and physics problems (*8*). In contrast, females score higher on tests of emotion recognition (*9*), social sensitivity (*10*), and verbal fluency (*11*). They start to talk earlier than boys do (*12*) and are more likely to play with dolls as children (*7*). Effect sizes range from small (Cohen's $d = 0.2$ for emotion recognition) to large (Cohen's $d = 1.3$ to 1.9 for targeting), with a substantial degree of overlap between male and female distributions, even for effects considered large by the conventions of psychology. All of these differences exist at the level of populations, not individuals; from such population differences, no inferences can or should be made about individuals.

Although these population differences partially arise from experiential factors, experiments in animals suggest a biological foundation. Male rats perform significantly better than females do on the radial arm and Morris water maze (*13*). This sex difference is eliminated by castrating males or by treating females with testosterone neonatally (*14*).

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Human males also commit fewer errors and require less time to complete a "virtual" maze (*15*). Young male vervet monkeys prefer to play with toy trucks, whereas young female vervets prefer dolls (*16*). This finding suggests that sex differences in toy preferences in children result, in part, from innate biological differences. Biological contributions to social interest are suggested by studies of human infants. When 1-day-old babies are presented with either a live face or a mechanical mobile, girls spend more time looking at the face, whereas boys prefer the mechanical object (*17*).

According to the empathizing-systemizing (E-S) theory of psychological sex differences, such differences reflect stronger systemizing in males and stronger empathizing in females (*18*). Systemizing is the drive to analyze a system in terms of the rules that govern the system, in order to predict the behavior of the system. Empathizing is the drive to identify another's mental states and to respond to these with an appropriate emotion, in order to predict and to respond to the behavior of another person. (Other people's emotional states and behavior cannot easily be predicted and responded to using systemizing strategies. Whereas a deterministic system given the same inputs always produces the same outputs, the input-output function of a person depends on subtle differences in current and past emotional context and is practically impossible to parameterize formally).

The E-S theory proposes that psychological sex differences are defined by the difference between the dimensions of empathizing (E) and systemizing (S), and it categorizes individual brain types as type S (S > E, more common in males), type E (E > S, more common in females), or type B (E = S, in those who are equally proficient at empathizing and at systemizing) (Fig. 1). Data from two questionnaires, the empathy quotient (EQ) and the systemizing quotient (SQ), reveal the existence of extreme types where S » E or E » S (Fig. 2), and SQ-EQ difference scores (Fig. 3) illustrate the differing profiles of the two sexes. Ongoing studies from our lab confirm the psychometric reliability and validity of these scales (*19*) and are evaluating how they correlate with performance tests (*20*).

Sex Differences in Brain Structure

Although there is a great deal of individual variance in human brain morphometry (*21*), it is known that the cerebrum as a whole is about 9% larger in men and is also larger in boys (*21*), a difference that is driven more by white matter than by gray (*22*, *23*). Despite the larger total volume of white matter in men [and despite the conflicting studies of sex differences in specific corpus callosum measures (*24*)], three-dimensional (3D) morphometry suggests that the ratio of corpus callosum to total cerebral volume is actually smaller in men (*22*). This is consistent with the findings that increased brain size predicts decreased interhemispheric connectivity (*25*) and that larger brains come with proportionately smaller corpora callosa in humans (*26*) and other species (*27*). Reports of anatomically localized cerebral sexual dimorphism are less consistent (*28*), but the male amygdala undergoes an extended period of growth during childhood (*29*); it is larger in boys (*30*) and may remain larger in men (*28*). These anatomical differences likely result from differences in microarchitecture. There are more neurons in the male cerebral cortex (*31*), and in general, these neurons are more densely packed (*32*), albeit with some regional exceptions (*33*).

Overall, greater numbers and denser packing of neurons, together with more intrahemispheric white matter projecting from these neurons, indirectly suggest a pattern of increased local connectivity and decreased interhemispheric (or long-range) connectivity in the male brain. Physiological observations, though sparse, seem consistent with this picture; language-related activation in female brains is more bilateral, suggesting greater interhemispheric connectivity (*34*, *35*), and the single study of gamma-band magnetoencephalography (MEG) reports increased phase locking between frontal and parietal sites in women during cognitive performance, again suggesting greater long-range connectivity (*36*).

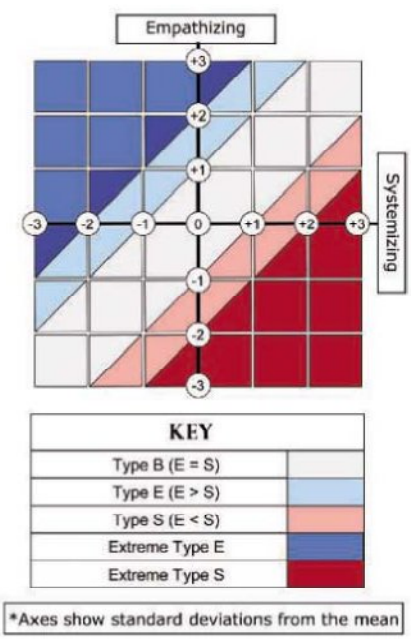

Fig. 1. The Empathizing-Systemizing model of sex differences at the psychological level.

The EMB Theory of Autism at the Psychological Level

An extension of the E-S theory of typical sex differences is the "extreme male brain" (EMB) theory (*37*). This theory proposes that individuals on the autistic spectrum are characterized by impairments in empathizing alongside intact or even superior systemizing. Adults with AS are more likely to have a brain of extreme type S (Fig. 2) and are distinguished by their high SQ-EQ difference scores (Fig. 3) (*38*). Table 1 gives the frequencies of all E-S brain types in the general population and in people with AS.

Reduced empathy in people with AS is evident in their lower scores on emotion-recognition tests (*39*), the EQ (*40*), the friendship and relationship quotient (*41*), and tests of social sensitivity such as the "faux pas" test (*10*). Intact or even superior systemizing is seen in their higher scores on the SQ (*42*), tests of folk physics (*43*), and the embedded figures test (*44*) (although it is unclear if the latter is really a test of systemizing or simply a test of good attention to detail). It is also seen in their strong obsessions, or areas of narrow interest, which tend to focus on systems (*45*).

It is clear how the EMB theory might characterize people with AS, but to what extent does the EMB theory apply to the whole autistic spectrum? People with classic autism have empathy deficits, or degrees of "mind blindness," in that they are delayed in developing a "theory of mind" in childhood and joint attention in infancy (*46*). It is less straightforward to test systemizing in someone with little language or with a below-average intelligence quotient (IQ). Nevertheless, characteristic behaviors such as "insistence on sameness," repetitive behavior, obsessions with lawful systems (e.g., train timetables),

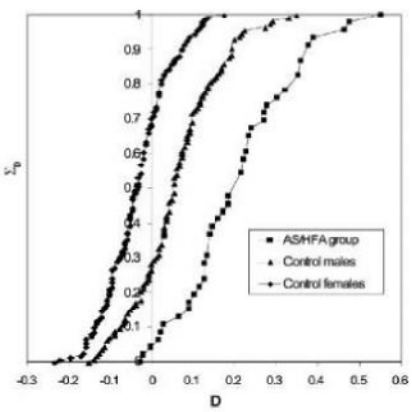

Fig. 2. Cumulative distribution function (Σ_D) of difference scores (D). This graph shows that the values of D between EQ and SQ significantly differentiate the three populations [males, females, and individuals with a diagnosis of AS/high-functioning autism (HFA)] (*82*).

islets of ability (e.g., calendrical calculation), precocious understanding of machines, and superior attention to the detection of change all involve a strong interest in rule-based prediction and therefore can be read as signs of hypersystemizing. It is unclear whether the risk of reduced IQ or language difficulties increases as systemizing becomes so strong that attention is narrowed to understanding just one unique system, making generalization of knowledge irrelevant (*47*). Of course, such symptoms may reflect other processes than systemizing, and competing hypotheses need to be tested.

The EMB Theory of Autism at the Neuroanatomical Level

Recent hypotheses concerning neural connectivity in the autistic brain postulate an exaggerated version of what may also be going on in the typical male brain: a skewed balance between local and long-range connectivity (*48*–*51*). Such a connectivity difference could give rise to a deficit in empathizing, because empathy activates brain regions that integrate information from multiple neural sources (*52*). In autism, furthermore, long-range connectivity during an empathizing task is abnormally low (*53*). This notion of skewed connectivity is also compatible with strong systemizing, because systemizing involves a narrow attentional focus to local information, in order to understand each part of a system. Imaging studies are needed to confirm this relationship.

Young children with autism tend to have larger-than-average heads. Magnetic resonance imaging morphometry confirms that these large heads contain abnormally large brains, an increase driven more by white matter than by gray (*54*). Although not yet confirmed by in vivo tract tracing, the anatomical distribution of this white-matter hyperplasia suggests it occurs more in short-distance tracts, whereas the internal capsule and corpus callosum are proportionately reduced (*55*–*57*). The development of the amygdala in autism likewise seems an extreme of typical male brain development. In children with autism between 18 and 35 months old, the amygdala is abnormally large, even when corrected for total brain volume (*58*). This enlargement persists through early childhood (*59*, *60*), exactly during the period of sex-differential amygdala growth in normal boys. By the time children with autism reach adolescence, the enlargement has disappeared (*60*); by early adulthood, the amygdala in autism is abnormally small (*61*, *62*).

Like an exaggeration of typical males, children with autism show enlargement of the cerebral cortex that stems more from white matter than from gray and may affect short-distance more than long-distance tracts. Again like an exaggeration of typical boys, children with autism also show greater growth of the amygdala. Future research will need to map all aspects of autistic neuroanatomy that are hypermasculinized, as well as consider how to explain those aspects that are not.

Prenatal Androgens Produce Sex Differences in Brain and Behavior

Which biological mechanisms shape the sex differences described above and may be pushing the autistic brain to develop beyond that of the typical male? In this section we review evidence for prenatal androgens as a key biological mechanism. Androgens, including testosterone produced by the testes in fetal and neonatal life, act on the brain to produce sex differences in neural structure and function. Testosterone is a small lipophilic molecule that easily passes through the blood-brain barrier and across cell membranes. The androgen receptor (AR) is a classic steroid receptor found in the cytoplasm. Once bound to testosterone (or its metabolite dihydrotestosterone), the AR enters the nucleus, where it binds DNA and affects transcription. Testosterone can also be aromatized to estradiol within the target cell, binding to the estrogen receptor (ER-α or ER-β) and influencing transcription similarly. Testosterone affects neural development by averting programmed cell death, influencing neural connectivity, and altering neurochemical profiles (*14*). For example, testosterone and estradiol modulate serotonergic and γ-aminobutyric acid neurotransmission, and they increase the formation of dendritic spines in a process mediated by brain-derived neurotrophic factor (BDNF).

In the fetal primate brain, substantial AR binding is observed in the cerebral cortex, cerebellum, mediobasal hypothalamus, amygdala, corpus callosum, and cingulate cortex of both sexes. Detectable levels of enzymes that convert testosterone to its active metabolites are also found in these regions (*63*). ER-α is found in the hypothalamus and amygdala, with lower concentrations also in the cerebral cortex (*64*). ARs are present as early as the first trimester, with high ex-

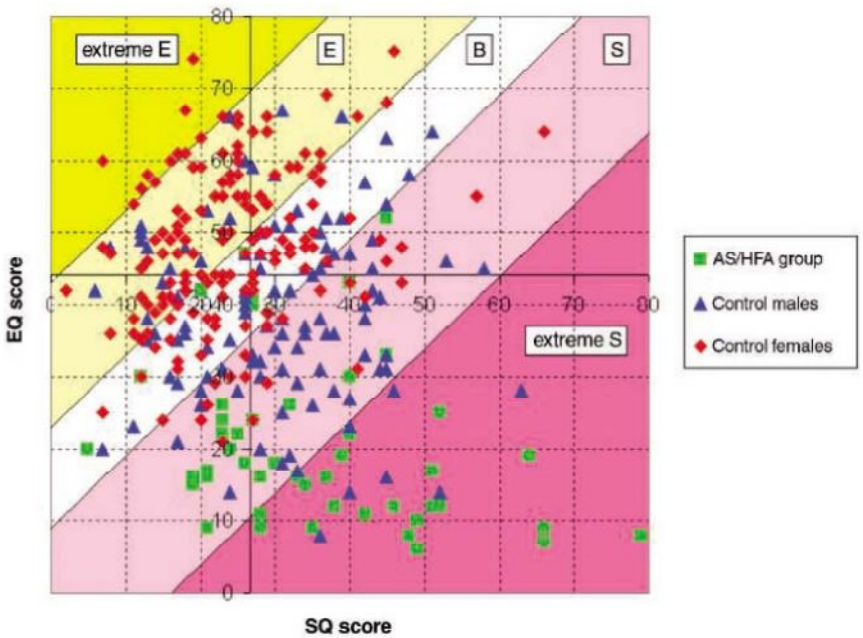

Fig. 3. SQ scores versus EQ scores for all participants, with the boundaries for the different brain types (*82*).

Table 1. Classifications of brain type based upon percentiles (*82*).

Brain type	Extreme E	E	B	S	Extreme S
Brain sex	Extreme female	Female	Balanced	Male	Extreme male
Defining characteristic	S « E	S < E	S ≈ E	S > E	S » E
Percentile (per)	per < 2.5	2.5 ≤ per < 35	35 ≤ per < 65	65 ≤ per < 97.5	per ≥ 97.5
Female %	4.3	44.2	35.0	16.5	0
Male %	0	16.7	23.7	53.5	6.1
AS/HFA %	0	0	12.8	40.4	46.8

pression in temporal cortex and other regions (*65*). AR binding in the developing cerebral cortex is higher in the right frontal lobe and the left temporal lobe in males, an asymmetry that is not present in females (*66*). Rats show a sexually dimorphic asymmetry in cortical thickness, dependent on testosterone and possibly related to receptor distribution. Although the literature on anatomical and functional asymmetries in humans is contentious, a number of researchers have suggested that the male brain is more strongly lateralized than the female brain (*67*). Although information on AR distribution in the human fetal brain is limited, AR distribution may be conserved across species. The single study of ER distribution in the human midgestational fetus shows ER-β but no ER-α expression in cortex (*68*).

In humans, exposure to atypically high levels of prenatal androgens results in masculine behavior and ability patterns (*69*). For example, females with congenital adrenal hyperplasia (CAH), a genetic condition that elevates fetal testosterone (FT), show tomboy behavior (*70*). Normal interindividual variation in prenatal hormone levels, measured in amniotic fluid, correlates with later sex-typed behavior (*71*–*74*).

All the sexually dimorphic brain regions discussed previously are rich in ARs, and their development therefore may be rather directly affected by testosterone (*28*), either early in fetal life or later. This raises the following question: If autism is an extreme of the male brain, is this the result of elevated FT, abnormalities in ARs or the genes controlling FT, or sexually dimorphic gene expression unrelated to FT? Currently, there are six clues that FT may play a role in autism: (i) FT is associated with low ratios of second-to-fourth digit length (*75*), and a low digit-length ratio is in turn associated with autism-spectrum conditions (*76*). (ii) Girls with CAH manifest more autism-like traits than their unaffected sisters (*77*). (iii) Within normal development, FT is inversely correlated with behaviors that, in the extreme, would count as diagnostic symptoms for autism. These are eye contact, vocabulary development, social functioning, and narrow interests (*72*–*74*). (iv) There is preliminary evidence of somatic hypermasculinization in autism, although a comprehensive study of this is needed (*78*). (v) There is precocious puberty in boys with autism. (vi) Serotonin levels (*50*) and BDNF levels are elevated in autism (*67*), and these are mediated by FT. A direct test of the FT hypothesis using amniocentesis is under way in our laboratory.

Further Work

Investigation of the EMB theory of autism demands more detailed normative data, especially in the areas of histology and physiology. Does network architecture differ between the sexes, and if so, in what ways? What can diffusion tensor imaging reveal about sex differences in white-matter topography? What will the application of new methods of functional connectivity analysis reveal about normal sex differences in functional imaging and quantitative electroencephalography (EEG) and MEG? Do males with more "female" E-S profiles have more "female" brain anatomies, and vice versa? And how do these differences in brain structure and dynamics change during development?

In parallel, the correlation between autism and exaggerated male brain characteristics can be explored by detailed anatomic study of regions that are known to be sexually dimorphic in the normal brain but that have not yet been investigated in the autistic brain, such as the interstitial nuclei of the anterior hypothalamus (*79*). In addition, it will be important to distinguish brain dimorphisms mediated by testosterone from those that arise more directly from genetic factors or those that depend on experience. Evidence for direct genetic effects on brain sexual dimorphism does exist. For example, mice in which chromosomal sex and gonadal sex do not correspond differ behaviorally in maze learning and neurochemically in vasopressin innervation of the lateral septum (*14*). Because 15% of X-chromosome genes escape X inactivation in humans (*80*), X-chromosome gene-dosage effects may play a role in such direct genetic effects. Neuroanatomical observations in populations with anomalous sex-chromosome variations may prove informative. In addition, it has been suggested that an imprinted X locus may explain sex differences in social and communicative skills and the male vulnerability to social and communicative impairments (*81*).

How the EMB theory applies to females with autism is also of interest. If a male brain is a risk factor for autism, this may explain the lower prevalence in females. If the EMB theory does apply to autism, might it apply more broadly to a range of neurodevelopmental conditions that affect males more than females? Lastly, even if the EMB theory can explain some core characteristics of autism, it will be important to establish which other comorbid characteristics require different explanations.

Conclusion

The EMB theory was first formulated by Hans Asperger as a clinical anecdote more than 60 years ago. In the past decade, it has been reformulated to be psychologically testable. Using psychometric definitions of the typical male and female brain, we have observed that people with autism-spectrum conditions show an exaggeration of the male profile. Evidence reviewed above suggests this may also apply to aspects of autistic neuroanatomy. The challenge ahead will be to test this theory across the whole autistic spectrum.

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Synthesis and Structure of Sila-Adamantane

Jelena Fischer, Judith Baumgartner, Christoph Marschner*

Bulk silicon is the central material of the modern semiconductor industry. Research into device miniaturization and molecular electronics (*1*) has therefore provoked interest in the changing properties that accompany the transition from a molecular silicon compound to its extended solid lattice. However, the preparation of molecules corresponding to substructures of the silicon crystal lattice has proven challenging.

The bulk silicon lattice adopts the same structure as diamond. In the case of diamond, the carbon skeleton of the smallest discretely defined lattice repeat unit can be isolated as the hydrogen-capped adamantane molecule. Here we report the synthesis of a silicon analog of adamantane, comprising the Si_{10} lattice building block capped by methyl and trimethylsilyl groups (Scheme 1).

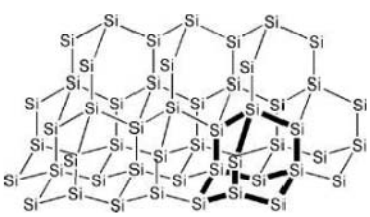
In general, past synthetic routes to silicon cluster compounds have employed the salt elimination reaction of trihaloalkylsilanes with alkali metals to yield $(RSi)_n$ structures. Tuning the size of the alkyl group has allowed selective preparation of various silaenbanes, -prismanes, and -tetrahedranes (*2*, *3*). However, cages with mono- and di-alkylated secondary Si centers have been more elusive. For example, attempts to react mixtures of dimethyldichlorosilane and methyltrichlorosilane with lithium in order to obtain cagelike molecules yielded only 3.3% permethylated bicyclo[2.2.2]octasilane and 4.4% bicyclo[3.3.0]decasilane, accompanied by 41.6% dodecamethylcyclohexasilane (*4*). No evidence of permethylated sila-adamantane, a possible product of this reaction, was found in this case.

We chose instead to pursue a Lewis acid–catalyzed rearrangement strategy, inspired by von Schleyer's route to adamantane (*5*). In contrast to the rearrangement of hydrocarbons, which leads preferentially to tertiary carbon centers, the analogous process in organosilicon chemistry is known to provide access to fourfold silylated silicon atoms (*6*). Given this thermodynamic preference for quarternary silicon centers, we set out to prepare a fourfold silylated sila-adamantane.

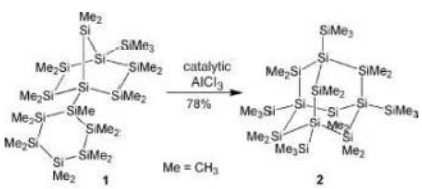
As adamantane is a tricyclic molecule, it was necessary to choose a tricyclic precursor. Application of recently developed silylpotassium chemistry (*7*) afforded a 1,4- disilylated bicyclo[2.2.1]heptasilane. Reaction of this compound with potassium *tert*-butoxide followed by undecamethylcyclohexasilanyl bromide yielded the desired precursor **1**, which rearranged on treatment with aluminum trichloride in cyclohexane to the desired sila-adamantane structure **2** in 78% yield (Scheme 2). The rearrangement entails conversion of dimethylsilylene units to trimethylsilyl groups and to quaternary silicon centers.

In solution, ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra of **2** showed the expected sets of two inequivalent methyl resonances. The ^{29}Si NMR spectrum showed three resonances at chemical shifts of –4.8, 26.0, and 118.6 parts per million (ppm). Compared to tetrakis(trimethylsilyl)silane (with shifts at 9.4 and 135.6 ppm) and dodecamethylcyclohexasilane (at –41.7 ppm), all resonances of **2** were considerably shifted downfield. Ultraviolet and visible spectra of **2** showed a strong absorption at 222 nm [molar absorption (ε) = 1.2×10^5 L mol^{-1} cm^{-1}], which is blue-shifted relative to other molecules containing pentasilane units (*8*).

The solid-state structure of **2** was confirmed by single-crystal x-ray diffraction analysis (Fig. 1). The compound appears to be completely unstrained. Si Si bond lengths were ~2.36 Å, and all bond angles around the quaternary silicon atoms were close to the tetrahedral ideal of 109.5° (108.8° to 110.1°). The torsional angles along the chain from one trimethylsilyl group to another indicate an almost perfect, all-*anti* conformation. This stereochemical arrangement is thought to be optimal for the delocalization of sigma-bond electrons along the chain (*9*).



Scheme 1.



Scheme 2.

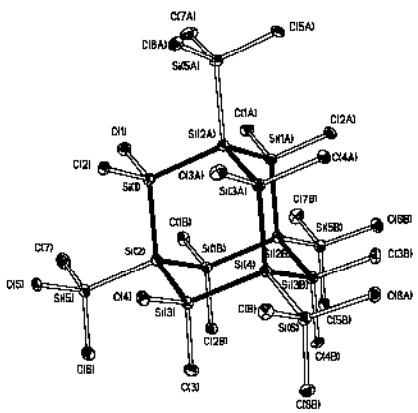


Fig. 1. Molecular view of **2** in the solid state.

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Structures of the Bacterial Ribosome at 3.5 Å Resolution

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We describe two structures of the intact bacterial ribosome from *Escherichia coli* determined to a resolution of 3.5 angstroms by x-ray crystallography. These structures provide a detailed view of the interface between the small and large ribosomal subunits and the conformation of the peptidyl transferase center in the context of the intact ribosome. Differences between the two ribosomes reveal a high degree of flexibility between the head and the rest of the small subunit. Swiveling of the head of the small subunit observed in the present structures, coupled to the ratchet-like motion of the two subunits observed previously, suggests a mechanism for the final movements of messenger RNA (mRNA) and transfer RNAs (tRNAs) during translocation.

Protein biosynthesis occurs on the ribosome in all forms of life. Ribosomes in bacteria are 21-nm particles composed of a small (30*S*) and a large (50*S*) subunit that associate to form the intact 70*S* ribosome (*1*). In contrast to most cellular machines, the ribosome contains a functional core of RNA that is enhanced by ribosomal proteins and accessory factors. All ribosomal functions rely in large measure in some cases entirely on ribosomal RNA (rRNA). In particular, rRNA is responsible for catalyzing peptide bond formation (*2*, *3*) and contributes to mRNA decoding and to mRNA and tRNA translocation after peptide bond formation (*4*–*6*).

Atomic-resolution structures of the 30*S* and 50*S* ribosomal subunits have provided insight into the mechanism of protein synthesis (*1*, *6*, *7*). However, protein synthesis occurs only in the context of the intact ribosome. Initiation of translation generally begins with mRNA start codon recognition and initiator tRNA binding to the small subunit. Subsequently, the large subunit associates with the small-subunit complex, and the elongation cycle begins (*1*). During the elongation cycle, the ribosomal subunits maintain a delicate balance of stable interactions with a large degree of flexibility. The structure of the translating ribosome has been modeled by comparing atomic-resolution structures of the 30*S* and 50*S* subunits (*8*–*11*) with low-resolution x-ray crystal structural models and several cryo–electron microscopic (cryo-EM) reconstructions of the *E. coli* 70*S* ribosome and of the yeast and mammalian 80*S* ribosome (*12*–*29*). Although the low-resolution structures reveal many of the large-scale motions in the ribosome that occur during the elongation cycle, the underlying molecular mechanisms that control these motions remain unknown.

We now describe two structures of the intact 70*S* ribosome from *E. coli* at a resolution of 3.5 Å, based on crystals that contain two independent copies of the ribosome per asymmetric unit. Intriguingly, the two ribosomes in the crystal adopt strikingly different conformations that may relate to the trigger of mRNA and tRNA movements on the small subunit during translocation (*30*, *31*). The structures also reveal a high degree of solvation at the subunit interface that may facilitate intersubunit movement (*16*). Finally, relative to isolated large subunits, the structures of the intact ribosome exhibit differences in the peptidyl transferase center that may reflect the dynamics necessary for rapid peptide bond formation.

Structure determination. *E. coli* 70*S* ribosomes, purified as described (*14*) and depleted of ribosomal protein S1 (*32*), formed crystals that contained two unique copies of the ribosome and diffracted x-rays to beyond 3.5 Å resolution (table S1) (*33*). Structure factor phases were initially obtained by molecular replacement using a 9 Å resolution model of the 70*S* ribosome derived from atomic-resolution structures of the 30*S* and 50*S* subunits (*14*). The resulting model for two ribosomes was then refined against 3.5 Å structure factor amplitudes by iterative rounds of torsional dynamics and manual rebuilding (table S1) (*33*). Differences in the structures of the two ribosomes limited the use of non-crystallographic restraints in the refinement to domains within the small and large ribosomal subunits (table S2) (*33*).

The present model consists of two ribosomes in which the rRNAs (16*S* rRNA, helices h1 to h45, in the small subunit; 23*S* rRNA, helices H1 to H101, and 5*S* rRNA in the large subunit) and ribosomal proteins (S2 to S21 and L2 to L36 in the small and large subunits, respectively) are modeled with *E. coli* sequence (*34*, *35*). Because refinement of the large-subunit proteins is ongoing, the results presented here include only those large-subunit proteins for which the modeling is nearly complete. At 3.5 Å resolution, the RNA backbone is visible and bases can be distinguished at the level of purines and pyrimidines in many regions of the structure. Ribose puckers have been modeled on the basis of stereochemical constraints on the allowed torsional angles in nucleotides and their fit to electron density maps. The proteins are generally resolved at the level of the backbone, and protein side chains are evident in well-ordered regions. In addition, hydrated magnesium ions appear clearly in most parts of the electron density map. The model contains more than 170 Mg^{2+} ions per ribosome.

Figure 1 shows the overall structure of one of the ribosomes in the "standard" view (Fig. 1A; 30*S* subunit in front, 50*S* subunit to the rear) and in a "top" view (Fig. 1B). Recognizable features of the small subunit include the head, body, shoulder, platform, and spur. On the large subunit, the main features include the L1 arm, consisting of protein L1 and its 23*S* rRNA binding site; the central protuberance (CP); the A-site finger (ASF) RNA helix; and the region near proteins L7/L12, which includes the L11 arm, consisting of protein L11 and its 23*S* rRNA binding site. Nearly all of the rRNA and proteins are visible in the electron density. The structural elements that are not fully modeled because of disorder include regions that are known to be highly mobile within the ribosome (*21*, *27*, *36*): most of the L1 arm (L1 in Fig. 1), proteins L10, L7/L12, the end of the ASF, the free end of the L11 arm, and the N and C termini of some of the ribosomal proteins.

Rotation of the 30*S* subunit head and implications for tRNA movement. The two ribosomes in the crystallographic asymmetric unit (termed ribosomes I and II) adopt different conformations primarily as a result of rigid-body motions of domains within the subunits. The most striking difference is that the head of the small subunit has swiveled as a rigid body around the neck region by 6° in the direction of the exit tRNA binding site (E site) in ribosome II when compared to ribosome I

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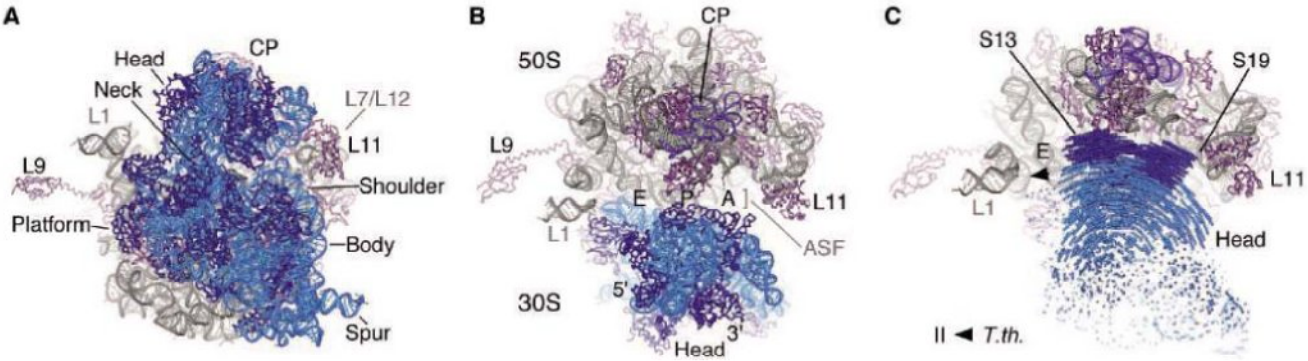

Fig. 1. Structure of intact *E. coli* 70S ribosome. **(A)** View from the solvent side of the 30S subunit. rRNA and proteins in the 30S subunit are colored light blue and dark blue, respectively. 23S rRNA and proteins in the 50S subunit are colored gray and magenta, respectively. 5S rRNA is colored purple. 30S features include head, neck, platform, body, shoulder, and spur. 50S features include L1 (protein L1/rRNA arm), CP (central protuberance), ASF (A-site finger), and L11 (protein L11/rRNA arm). The approximate location of proteins L7/L12 and the tip of the ASF, not observed in the structure, are in gray. **(B)** View rotated 90° about the horizontal axis in (A). Letters indicate the approximate alignments of the aminoacyl (A), peptidyl (P), and exit (E) tRNA binding sites at the subunit interface. The 5′ to 3′ direction of mRNA, which threads around the neck region of the 30S subunit, is also indicated. **(C)** Position of the head domain of the 30S subunit in ribosome II compared to the *T. thermophilus* 70S ribosome. Differences in the position of corresponding phosphorus atoms (light blue) and Cα positions for S13 and S19 (dark blue) in the two head domains are shown as vectors pointing in the direction of the arrows. The view is 60° about the horizontal axis in Fig. 1A.

(fig. S1) (*33*). In turn, the position of the head of the small subunit in ribosome I is rotated toward the E site by 6° when compared to the 5.5 Å structural models of the *Thermus thermophilus* 70S ribosome, which contain mRNA and tRNAs bound in the A and P sites (*15*) or in the P and E sites (*12*), respectively (fig. S1). Thus, the total rotation of the head that is observed in the three structures is about 12°, or 20 Å at the subunit interface (Fig. 1C).

The rotation of the head domain of the small subunit seen in the present structures parallels the trajectory of tRNAs through the ribosome and may provide a mechanism for controlling mRNA and tRNA movement during translocation. After peptide bond formation, the tRNAs move from the A and P sites in the pretranslocation state to the P and E sites in the posttranslocation state. The pretranslocation tRNAs occupy a hybrid state of binding (A/P and P/E sites) at least transiently before translocation is complete (Fig. 2A) (*37*). Recent biochemical evidence also suggests that mRNA and tRNA translocation requires an "unlocking" step on the small subunit (*30*, *31*, *38*). Peptide bond formation allows tRNA movement on the large subunit and permits a ratcheting motion of the subunits required for translocation (*16*, *21*, *27*, *38*, *39*) (Fig. 2B). Spahn *et al.* (*27*) proposed that subsequent rotation of the head of the small subunit (Fig. 2B) directs movement of the tRNAs to the P and E sites. However, the structural basis for the "unlocking" event remains unclear.

The present structures provide a detailed view of changes in the small subunit that allow swiveling of the head, and also suggest a mechanism for "unlocking" the small subunit to complete translocation. In comparisons between ribosomes I and II and the *T. thermophilus* 30S subunit, nearly all of the conformational changes responsible for the rigid-body rotation of the head are concentrated in four base pairs (nucleotides 929 to 932 paired with 1388 to 1385) in h28 of 16S rRNA (Fig. 2C) (*33*). It is noteworthy that six of the seven base pairs in helix h28 are G-C base pairs in most bacteria (fig. S2). This high G-C content is highly conserved across all kingdoms (*34*), which suggests that helix h28 may act as a stiff spring to absorb the strains induced by the rotation of the head domain (*40*).

When compared to previous results (*8*, *12*, *15*), the present structures reveal that the "lock" that must be opened during translocation is likely a steric block between the P and E sites on the small subunit. In all of the available structures, the path that A-site tRNA traverses to enter the P site is relatively unobstructed on the small subunit. In contrast, residues G1338 to U1341 in 16S rRNA form a stable ridge in the head of the small subunit (fig. S2) that sterically separates the anticodon stem-loop of P-site tRNA from the E site (*12*, *15*). In a 5.5 Å structure of the pretranslocation state (*15*) and in an 11 Å structure of a P/E hybrid state (*21*), the G1338-U1341 ridge in the head and A790 in the small-subunit platform leave a gap of only 12 to 13 Å (fig. S2); more than 20 Å would be needed for an A-form RNA helix to pass through. This "lock" in the small subunit seems to allow tRNA movement to the P/E hybrid state, but prevents complete translocation (*21*, *31*). The observed rotations of the head domain of the small subunit in ribosomes I and II do not increase the gap, and the head domain would retain tight packing with the P-site tRNA anticodon stem (Fig. 2D). Interestingly, the conformation of the 30S subunit in ribosome II resembles that of the 40S subunit in the yeast 80S ribosome bound to elongation factor eEF2 and sardorin, with respect to rotation of the head domain (*27*). However, the conformation of the 40S subunit in the yeast 80S ribosome eEF2-sardorin complex opens the gap between the G1338-U1341 ridge and A790 to more than 20 Å, sufficient for P-site tRNA to move into the E site (Fig. 2E).

The mechanism for tRNA and mRNA translocation may therefore involve three types of movement within the small subunit: an overall ratcheting, swiveling of the head, and an opening of the tRNA binding groove to allow P-site tRNA to pass into the E site (Fig. 2B). Opening of the tRNA binding groove may represent unlocking of the small subunit, and this likely occurs physiologically only during or after guanosine triphosphate (GTP) hydrolysis by elongation factor EF-G (*21*, *30*, *31*). The precise ordering of these motions, however, will require new structural information about translocation intermediates.

Positions of the L1 and L11 arms in the 50S subunit. Two regions in the 50S subunit that change position as part of the translational elongation cycle are the L1 and L11 arms. The L11 arm contributes to the activity of initiation, elongation, and release factors (*18*, *19*, *21*, *23*, *25*, *41*, *43*), whereas the L1 arm is thought to influence movement of tRNA into and out of the E site (*12*, *21*). In the two *E. coli* ribosome structures, which lack bound tRNAs and translation factors, there are no contacts between these regions and the core of the ribosome that fix their respective positions. The different positions of the L1 and L11 arms in the two structures depend on conformational changes within RNA helices (fig. S3) (*33*). This means that three of the large-scale motions identified within the ribosome—movement of the L1 and L11 arms and swiveling of the head domain of the small subunit—depend on conformational changes

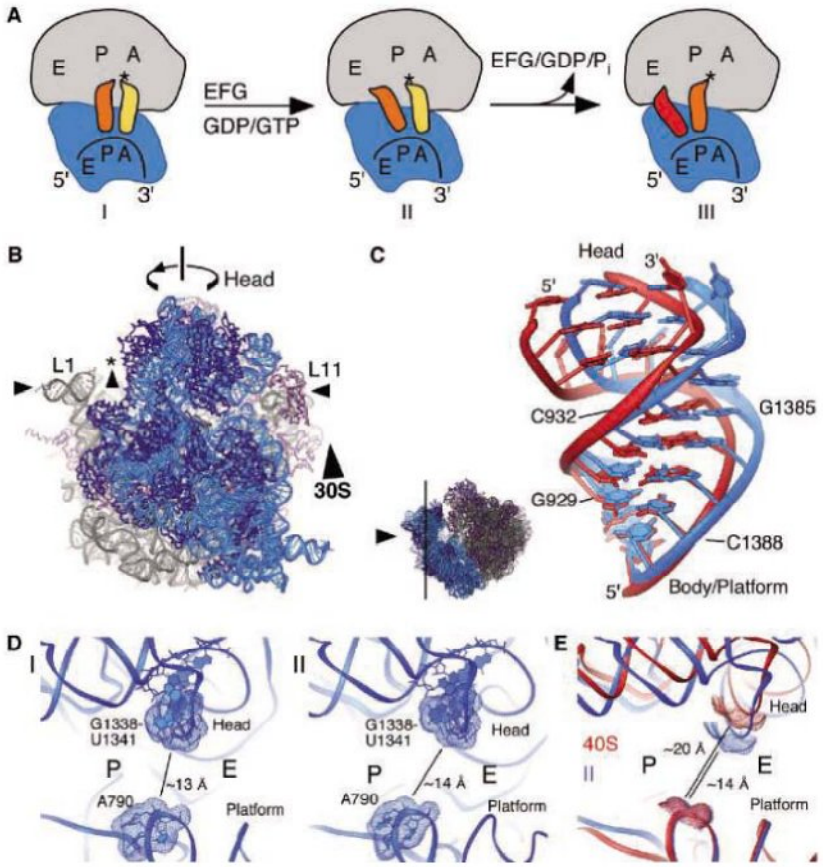

Fig. 2. Small-subunit tRNA binding sites and translocation intermediates. (**A**) Schematic steps in the mRNA and tRNA translocation reaction. After peptide bond formation, the pretranslocation complex (I) binds EF-G and shifts to the hybrid state of tRNA binding (complex II). Subsequent GTP hydrolysis by EF-G leads to the posttranslocation state (complex III). [Model abbreviated from (*31*)] (**B**) Model of structural changes in the ribosome that contribute to translocation, viewed as in Fig. 1A. Arrows indicate motions of the small and large subunit proposed to occur during the ratcheting mechanism: ratcheting of the small subunit, rotation of the small-subunit head domain, opening of the tRNA binding groove (asterisk), and lateral movements of the L1 and L11 arms (*16*, *21*, *27*). (**C**) View of the conformational differences in h28 of 16S rRNA in ribosomes I (blue) and II (red). The direction of view is indicated to the left. (**D**) View of the tRNA binding cleft in the 30S subunit, from the perspective of the large subunit. The G1338-U1341 ridge and A790, which separate the P and E sites, are marked. Molecular surfaces of the tRNA binding cleft in ribosome I (left) and ribosome II (right) are indicated. The shortest distance between the van der Waals surfaces is marked in each case. (**E**) Superposition of the 30S subunit in ribosome II (blue) with the yeast 40S subunit from the 80S-eEF2-sardorin complex (red) (*27*, *51*). Molecular surfaces and distances are shown as in Fig. 2D.

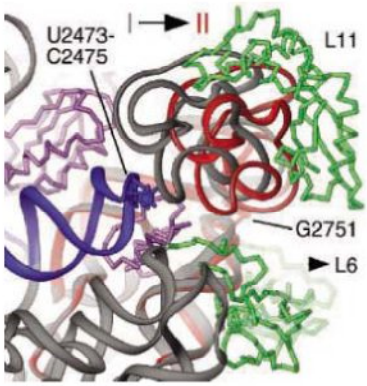

Fig. 3. Movement of the L11 arm in the two ribosomes. Nucleotides U2473 to C2475 in H89 and the tertiary contact with G2751 are marked. Proteins L6 and L11 from ribosome II are shown in green. Proteins L6 and L11 in ribosome I are not shown for clarity. The direction of movement of L6 is indicated by an arrowhead. The direction of view is that of Fig. 1A.

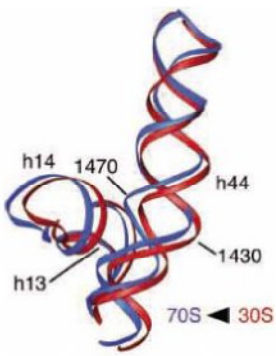

Fig. 4. Conformational changes in the 30S subunit when compared to the 70S ribosome. Helices h13, h14, and h44 in the 30S subunit (red) and 70S ribosome (blue) are shown from the interface side of the 30S subunit. Nucleotides and rRNA helices near the bend in h44 are marked.

within RNA helices, not on changes in tertiary structure.

When compared to ribosome II, the L11 arm in ribosome I has moved toward the tRNA binding sites at the subunit interface by about 15 Å (Fig. 3). This range of motion is the same as that observed in cryo-EM reconstructions of the yeast ribosome as part of the proposed mechanism of translocation (*27*). Similar but smaller conformational changes were observed in cryo-EM reconstructions of the *E. coli* ribosome in mRNA decoding intermediates (*18*, *22*). In the "closed" conformation observed in ribosome I, and also in the *T. thermophilus* 70S structures (*12*, *15*), loop nucleotides U2473 to C2475 at the end of H89 may act as a physical stop, preventing further movement inward toward the tRNA binding sites. On the other side, a tertiary interaction to G2751 in the loop of H97, also seen in the *Haloarcula marismortui* 50S structure (*10*), may limit outward movement of the L11 arm (Fig. 3) (fig. S3).

Comparisons to the 30S and 50S subunit structures. The overall conformations of the *H. marismortui*, *Deinococcus radiodurans*, and *E. coli* 50S subunits are remarkably similar (*33*). Apart from differences in the positions of the L1 and L11 arms, changes mainly reflect the mobility in 23S rRNA helices H69 and H34, and parts of proteins L2, L14, and L19 (*10*, *11*). These rRNA helices and proteins all reside in or near the subunit interface, as described below. By contrast, the 30S subunit (*8*) undergoes substantial rearrangement upon association with the 50S subunit (fig. S4). Conformational changes occur in the 30S subunit body and penultimate stem of 16S rRNA and in the relative position of the head and body domains with respect to the platform. The platform itself is nearly indistinguishable in the 30S and 70S structures (*33*). The head domain is also highly similar in the 30S and 70S structures (*33*), although, as noted previously, the head domain in each *E. coli* 70S ribosome is rotated toward the E site when compared to the 30S subunit.

Changes in the body of the small subunit when comparing 30S subunit structures to the 70S ribosome structures may be more relevant to the mechanism of translocation than to the mechanism of mRNA decoding. During mRNA decoding, the shoulder of the small subunit is thought to "close" around cognate, but not near-cognate or noncognate A-site tRNAs (*6*). The shoulder of the small subunit

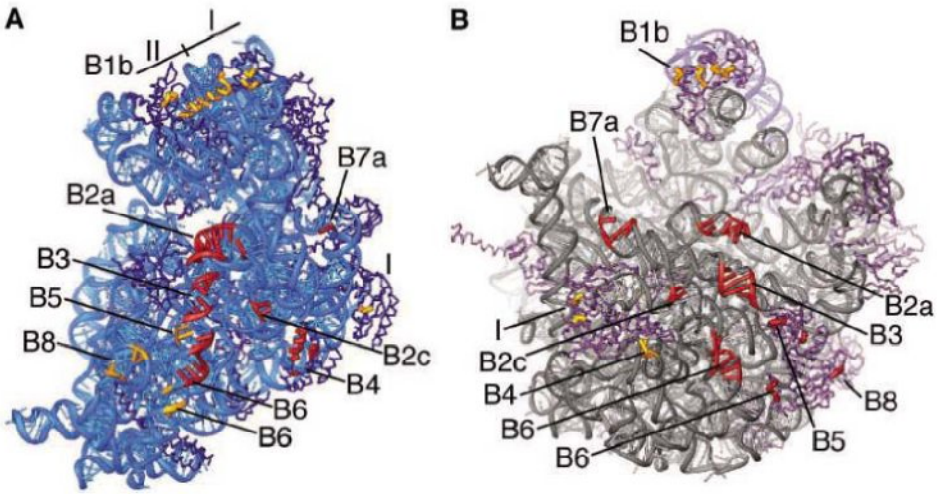

Fig. 5. Bridges between the 30*S* subunit and the 50*S* subunit. (**A**) Contacts at the interface of the 30*S* subunit, color-coded by type of interaction. Colors of rRNA and proteins are as described in Fig. 1A. Interactions that occur in only one of the two ribosomes are marked by ribosome (I or II). Interactions with protein in the opposite subunit, gold; interactions with RNA, red. (**B**) Contacts at the interface of the 50*S* subunit, color-coded by type of interaction as in (A).

in both 70*S* ribosomes adopts a conformation similar to that in the "open" 30*S* subunit structures (*6*), which suggests that subunit association has little effect on this structural rearrangement. By contrast, parts of helix h44 in 16*S* rRNA, the penultimate stem that runs along the body of the small subunit, and helices h8 and h14 are displaced laterally away from the platform side of the interface by 4 to 7 Å in the 70*S* ribosome structures when compared to the 30*S* subunit structure (fig. S4). The result is a penultimate stem that is less bowed in the 70*S* ribosome (Fig. 4). Helices h8, h14, and h44 are involved in a number of intersubunit contacts, as described below, which may explain the conformational differences. Consistent with this idea, the conformation of these helices in the *T. thermophilus* 70*S* ribosome structures seems to be the same as that seen here (*12*, *15*).

Interactions at the subunit interface. Previous structures of the ribosome have identified a number of intersubunit contacts, or bridges, that hold the ribosomal subunits together (*12*, *21*, *27*). In the present structures, the bridges at the subunit interface (Fig. 5) bury more than 6000 Å^2 of solvent-accessible surface area (*44*). However, a number of the intersubunit contacts have been proposed to rearrange, or even to break, as part of the elongation cycle (*12*, *21*, *27*). Apart from differences in the contacts to the head of the small subunit (described below), the bridges between the two ribosomal subunits at the interface are similar in the two structures of the *E. coli* ribosome. The RNA residues directly involved in subunit-subunit contacts superimpose with a root mean square deviation of less than 1 Å (*33*). When compared to the lower resolution ribosome structures, the interface between the body and platform of the 30*S* with the 50*S* subunit seen in the present structures adopts a conformation closest to that of the pretranslocation or posttranslocation state (complexes I or III in Fig. 2A) (*12*, *15*, *21*).

Two bridges between the head of the small subunit and the central protuberance of the large subunit in cryo-EM reconstructions are mirrored in the structures of the 70*S* ribosome presented here. In the first bridge [B1b/B1c in previous publications (*12*, *21*, *27*)], ribosomal proteins S13 and L5 contact each other. Protein S13 plays a key role in subunit association and in the fidelity of translocation because of its interactions with L5 in the central protuberance and with P-site tRNA (*8*, *21*, *45*–*47*). In both the pre- and posttranslocation states (*12*, *15*), L5 is directly across from the N-terminal globular domain of S13. In ribosome I, this contact is offset because S13 and the head of the small subunit are rotated toward the E site by several angstroms (Fig. 6A). The offset in ribosome I results in weak contact between S13, S19, and L5 (less than 400 Å^2 in buried surface area) (*33*), consistent with the dynamic nature of this interaction (*16*, *21*).

Strikingly, both the ratchet-like motion of the small subunit in the 70*S*/EF-G/GMPPNP [guanosine 5′-(β,γ-imido)triphosphate] complex (*21*) and the swivel motion of the head domain in ribosome II, although mechanically different, result in similar contacts between S13, S19, and L5. In ribosome II, the long α helix extending from the globular domain of S13 and running along the head of the small subunit forms a "rail" that lies in a shallow groove in protein L5 (Fig. 6B). Interactions between the three proteins are not extensive (<800 Å^2 buried surface area) (*33*), consistent with the observation that the contact rearranges during translocation (*16*, *21*).

The other bridge between the head of the small subunit and the large subunit, bridge B1a, includes the ASF (or H38 in 23*S* rRNA), which spans the subunit interface parallel to the A and P sites (Fig. 1B). This contact is not visible in the present structures because of disorder at the end of the ASF in both ribosomes I and II. Bridge B1a is clearly visible in the *T. thermophilus* ribosome structures and in post-translocation complexes imaged by cryo-EM (*12*, *15*, *21*). However, in an interesting parallel to the structures of ribosomes I and II, this contact is visibly weakened in the cryo-EM reconstruction of the EF-G/GMPPNP posttermination complex (*21*). The dynamics of the ASF contact due to rotation of the head of the small subunit and the ratchet motion may aid movement of A-site tRNA into the P site. The antibiotic viomycin, which inhibits translocation, protects nucleotides near the base of the ASF from chemical modification (*48*); this finding supports a role for the ASF in translocation.

Interpretations of cryo-EM reconstructions identify two possible pivot points on either side of intersubunit bridge B3 for the ratcheting of the small subunit relative to the large subunit (*21*, *27*) (Fig. 5). Bridge B3 contains the largest RNA-RNA minor-groove surface complementarity among the interface contacts, which may preclude large rearrangements in this bridge during translocation. Bridge B3 may therefore serve as the pivot point of the ratcheting motion. In bridge B3, two sheared G-A base pairs in h44 of 16*S* rRNA form a type I A-minor interaction (*49*) with two G-C base pairs in II71 of 23*S* rRNA in the large subunit (Fig. 6C) (fig. S5). As observed in other type I A-minor interactions, nearly all hydrogen bonds are satisfied by the close packing interaction. Energetic contributions to similar contacts within a group I self-splicing intron and during mRNA decoding suggest that surface complementarity helps to stabilize association, whereas hydrogen bonding compensates for desolvation (*50*, *51*).

At the apex of the small-subunit platform, bridge B7a (Fig. 5) involves the only cross-subunit base stacking interaction, between A702 in h23 of 16*S* rRNA and A1848 in H68 of 23*S* rRNA. In combination with A1848, A702 forms a variant of the A-minor motif (*49*) in which the adenosines pack against adjacent C-G base pairs in II68 of 23*S* rRNA (Fig. 6D) (fig. S5). Interestingly,

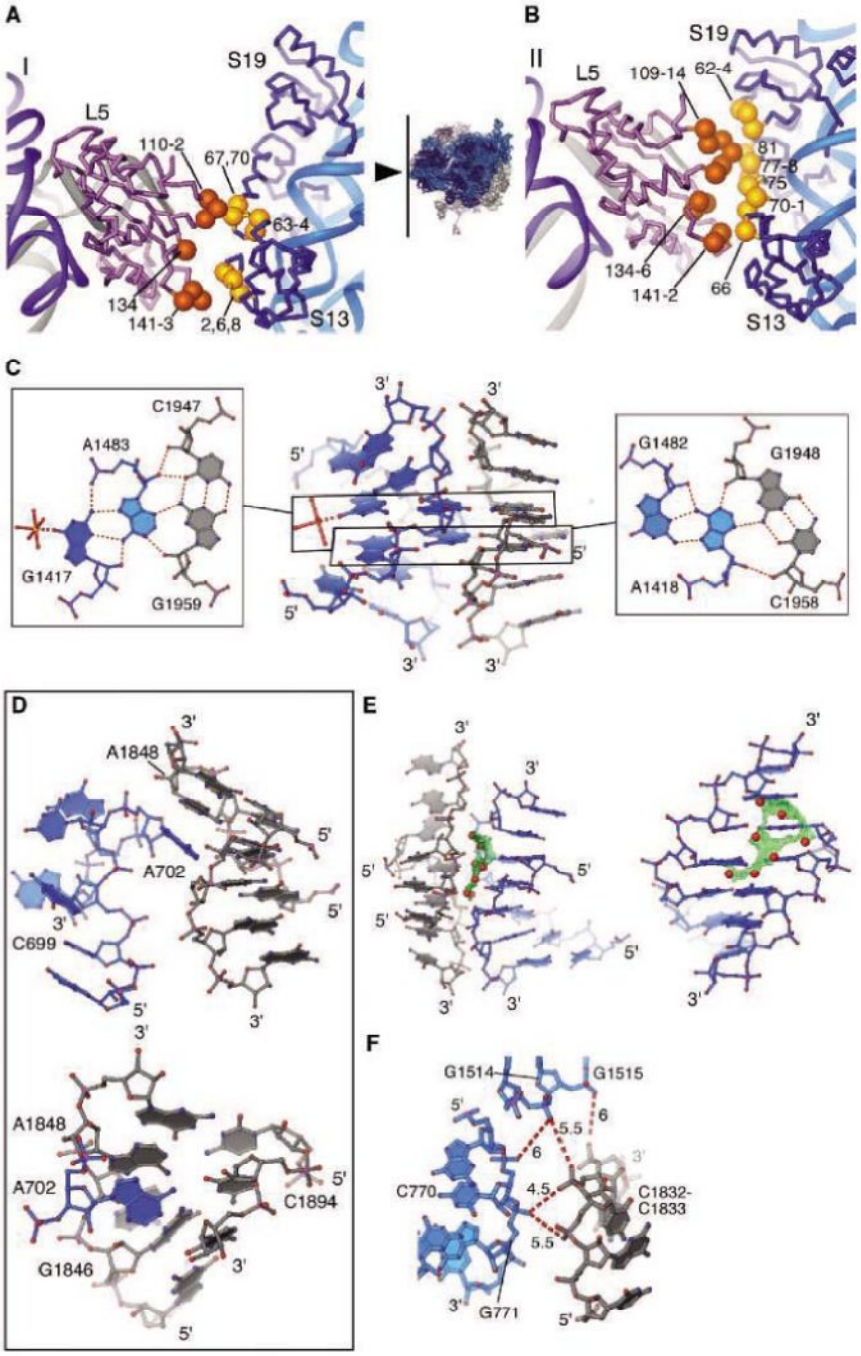

Fig. 6. Molecular interactions in the intersubunit bridges. **(A)** Contact between S13 and L5 in ribosome I. **(B)** Contact between S13 and L5 in ribosome II. Only the Cα traces for the proteins are shown, because protein side chains are not clear in the electron density of either ribosome. Residues that become inaccessible to solvent (*44*) are indicated in orange for L5 and in yellow for S13 and S19. The direction of view is indicated in the center. **(C)** Molecular interactions in bridge B3. **(D)** Molecular interactions in bridge B7a. **(E)** Molecular interactions in bridge B6. Waters modeled at the interface are shown as red spheres inside the water-accessible volume, green mesh. **(F)** Close approach of phosphates at the subunit interface near bridge B2c. Distances (in angstroms) between phosphate oxygens are marked.

the helical geometry in this region of 23*S* rRNA is conserved between archaea (*10*) and bacteria, whereas the residue at position 702 in 16*S* rRNA is kingdom-specific (*34*). In the ratchet-like motion (Fig. 2B), the interface in this region shifts by at least 6 Å laterally with respect to H68 (*21*, *27*), indicating that this contact must break during translocation.

In striking contrast to the desolvated nature of bridge B3, the neighboring bridge B6, between h44 in 16*S* rRNA and H62 in 23*S* rRNA, buries a large surface area that is almost entirely solvated. The minor grooves of h44 and H62 contact each other sparingly and leave a 6 Å gap that can accommodate a monolayer of water molecules (Fig. 6E) (fig. S5) (*33*). These water molecules can all be positioned within 3 Å of minor-groove hydrogen bond donors and acceptors in both subunits. Interestingly, many previously identified bridges between the center of the small-subunit platform and the large subunit are also highly solvated (*12*) (Fig. 5). There are many instances where phosphate groups from both subunits lie within 4 to 6 Å of each other (Fig. 6F), suggesting that nonspecific ions form part of the interface solvation network (*52*). Other bridges that contribute only small regions of direct contact in the present structures are described in (*33*). The high level of solvation at the subunit interface may be necessary to allow ratcheting during translocation, where the relative orientation of the two subunits may change by 7° to 10° (*21*, *27*).

Bridges B2a and B4 occur between the 30*S* platform and the 50*S* subunit (Fig. 5) and are essential for subunit association (*53*). Bridge B2a occurs at the functional center of the ribosomal interface and is immediately adjacent to the mRNA decoding site, between the top of h44 in 16*S* rRNA and H69 in 23*S* rRNA, and extends under the P site toward h45 and h24 (*12*, *18*, *22*). During mRNA decoding, the closing loop of H69 resides immediately below the D stem of A-site tRNA, whereas the adjacent stem of H69 is located below the D stem of P-site tRNA (*12*, *18*, *22*). H69 moves laterally with respect to the small subunit by 6 to 8 Å during the ratchet-like motion of the small subunit during translocation (*21*). Furthermore, ribosome recycling factor (RRF) causes the tip of H69 to peel away from the 30*S* subunit as part of the subunit dissociation process (*24*, *54*, *55*). The present structures provide a detailed view of H69 in the context of the 70*S* ribosome.

In the two *E. coli* ribosome structures, the base of H69 is identical in conformation through base pair Ψ1911/A1919. The loop at the end of H69 has slightly different conformations in the two ribosomes, possibly because of tighter packing at the interface between h44 and H69 in ribosome II when compared to ribosome I (fig. S5). However, a number of noncanonical base pairs in the loop that likely contribute to subunit association is conserved in the two structures. A widened reversed-Hoogsteen base pair between Ψ1911 and A1919, bridged by the 2′-OH of A1918, allows A1918 and A1919 to form an A-A dinucleotide platform (*56*) (Fig. 7A). This projects A1919 into the minor groove of h44 near bases U1406/U1495, where it also interacts with the base of

G1517 (Fig. 7A). Nucleotide A1912, which stacks on A1918 and forms a distorted reversed-Hoogsteen base pair with Ψ1917, projects into the minor groove of base pair C1407/G1494 in h44 of 16*S* rRNA (Fig. 7A). The involvement of all three N1 positions of A1912, A1918, and A1919 in packing interactions is consistent with interference of subunit association when these residues are N1-methylated by dimethyl sulfate (*53*). Other details of the bridge B2a interactions are given in (*33*).

Bridge B4 near the base of the platform likely remains intact even during the ratchet-like motion of translocation (*27*). Bridge B4 involves stem-loop H34 in 23*S* rRNA and protein S15 in the small subunit (*57*) (Fig. 5). H34 extends from the surface of the 50*S* subunit by about 30 Å and has been observed in different orientations in the isolated 50*S* subunit structures (*10*, *11*). This helix is nearly 60 Å from the putative pivot point of the ratchet-like motion between subunits (bridge B3, Fig. 5), which may explain the need for its flexibility in order to maintain intersubunit interactions. In the contact, the closing loop of H34 forms a U-turn in which A715 packs against a hydrophobic surface on S15 (Fig. 7B) (fig. S5). In addition, Gln^{59} and Arg^{88} of S15 interact with the minor and major grooves of the loop nucleotides, respectively. Methylation of the N1 position of A715 has been shown to interfere with subunit association (*53*). Interestingly, the N1 position of A715 is not in direct contact with any residue of either subunit, but is 4 to 5 Å from the guanidinium group of Arg^{52} in S15. The interference may therefore be due to a positive charge on 1-methyladenosine (*58*), which would lead to charge-charge repulsion with Arg^{52}.

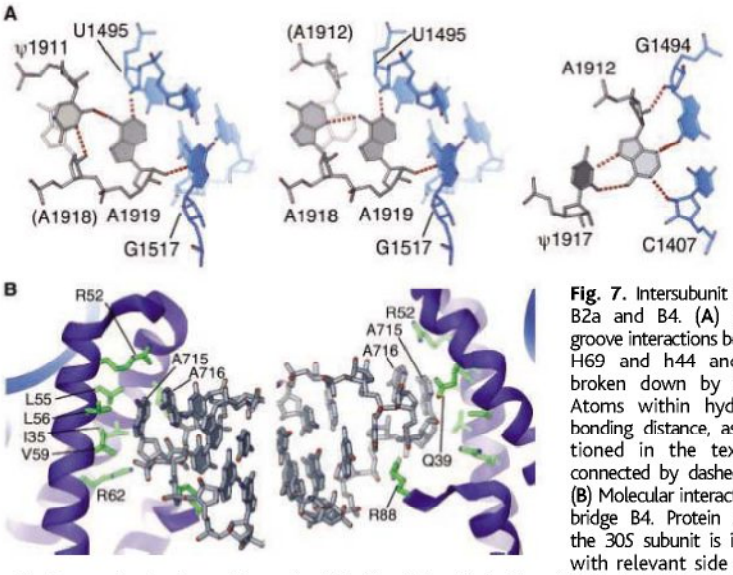

Fig. 7. Intersubunit bridges B2a and B4. (**A**) Minor-groove interactions between H69 and h44 and h45, broken down by region. Atoms within hydrogen-bonding distance, as mentioned in the text, are connected by dashed lines. (**B**) Molecular interactions in bridge B4. Protein S15 in the 30*S* subunit is in blue, with relevant side chains in green. The interaction is viewed from the left side of Fig. 5A (left) and from the right side of Fig. 5A (right). Electron density is visible for the side chain of Arg^{88} (R88) in ribosome II, but not in ribosome I. Other amino acid abbreviations: I, Ile; L, Leu; V, Val; A, Ala; Q, Gln.

Magnesium ion binding sites in the 70*S* ribosome. Formation of the 70*S* ribosome and the process of protein synthesis are highly dependent on the concentration of divalent metal ions in vitro (*59*–*61*). Some of the magnesium dependence is likely due to the close approach of phosphates at the interface, as described above (Fig. 6F) (*52*). Interestingly, most of the specific magnesium ion binding sites identified in the 30*S* subunit within the 70*S* ribosome are identical to those in the isolated 30*S* subunit (*62*) (fig. S7), despite sequence differences between the *T. thermophilus* and *E. coli* ribosomes and the conformational changes that occur upon subunit association (Fig. 4) (fig. S4). Comparisons to the *H. marismortui* 50*S* subunit reveal that about 40% of the Mg^{2+} binding sites are conserved across kingdoms and upon subunit association (*63*) (fig. S7). Most of the Mg^{2+} ions that are conserved involve one to four inner-sphere coordination sites to rRNA phosphate oxygens and electronegative groups on the bases (N7 of purines, O6 of guanosines, O4 and O2 of uridine, and O2 of cytidine). The networks of conserved Mg^{2+} binding sites indicate that magnesium ion cores are a conserved part of RNA tertiary structure within the ribosome (*63*, *64*).

Comparison with the 30*S* and 50*S* subunit structures reveals only one clear example of a specific magnesium ion binding site that becomes occupied when the subunits associate to form the 70*S* ribosome. This site occurs in the major groove of contact B3 (Fig. 6C). In the structure of the *T. thermophilus* 30*S* subunit, a fully hydrated Mg^{2+} site occurs adjacent to the tandem sheared G-A base pairs (*8*). Upon subunit association, this Mg^{2+} moves to adopt inner-sphere coordination with G1417 (Fig. 6C). The sequence of 16*S* rRNA near the ion is identical in *T. thermophilus* and in *E. coli* (*34*), ruling out sequence-dependent effects.

Conformation of the peptidyl transferase center. The peptidyl transferase center (PTC) of the ribosome is located in the large ribosomal subunit at the base of a cleft that binds the acceptor ends of tRNAs (*3*, *10*). Although isolated 50*S* subunits are active in peptide bond formation, the rate of catalysis on 50*S* subunits is lower than that of intact 70*S* ribosomes by a factor of about 1000 (*65*, *66*). The mechanism used by the ribosome to accelerate peptide bond formation is thought to be due almost entirely to substrate positioning within the active site (*67*, *68*), coupled to substrate-assisted catalysis (*69*). This suggests that conformational differences in the intact ribosome, when compared to isolated 50*S* subunits, may be responsible in large part for the differences in the observed rates of peptidyl transfer.

In the two 70*S* ribosomes, the PTCs are nearly identical in conformation (*33*). Comparison of the *E. coli* and *D. radiodurans* structures indicates that the overall conformation of the PTC is more closed in the 50*S* subunit than in the 70*S* ribosome, because of movement of both P-site and A-site residues toward the geometric center of the PTC (*11*) (Fig. 8A). In the *D. radiodurans* 50*S* subunit structure, the P-loop bases responsible for base pairing to the CCA end of P-site tRNA (G2251 and G2252), as well as nucleotides 2062 and 2063, are shifted laterally toward the A site, whereas nucleotides U2584 and U2506 on the A-site side are shifted toward the P-site side of the PTC.

The PTC of the *H. marismortui* 50*S* subunit is also more closed relative to that in the 70*S* ribosome (*3*, *5*, *33*, *70*). However, in this case the P-site side of the PTC is essentially identical, whereas in the *E. coli* 70*S* ribosome structures, nucleotide U2506 on the A-site side has shifted toward base A2451 at the side of the PTC, and U2584 is rotated away from A2451 (Fig. 8, B and C). A shift of U2506 toward A2451 would have a substantial impact on the positioning of A76 in the A-site tRNA, because of its interactions with the base pair between U2506 and G2583 in 23*S* rRNA. A76 would have to move toward the A2451 strand by 1.5 to 2 Å to avoid steric clashes and to form hydrogen bonds with the minor-groove face of the nucleotide (Fig. 8C). Note that the difference in conformation between the PTC in the *H. marismortui* 50*S* subunit and in the *E. coli* 70*S* ribosome does not seem to be driven by changes at the subunit interface. Instead, the position of the factor binding site region (H91 and H95 in 23*S* RNA) of the *H. marismortui* 50*S* subunit is moved toward the subunit interface, which in turn affects the position of the U2506 rRNA strand and the A-loop that forms a base pair with C75 of A-site tRNA (*3*, *5*, *70*–*72*).

In the 70*S* ribosome, some degree of flexibility in U2506 can be detected in one of

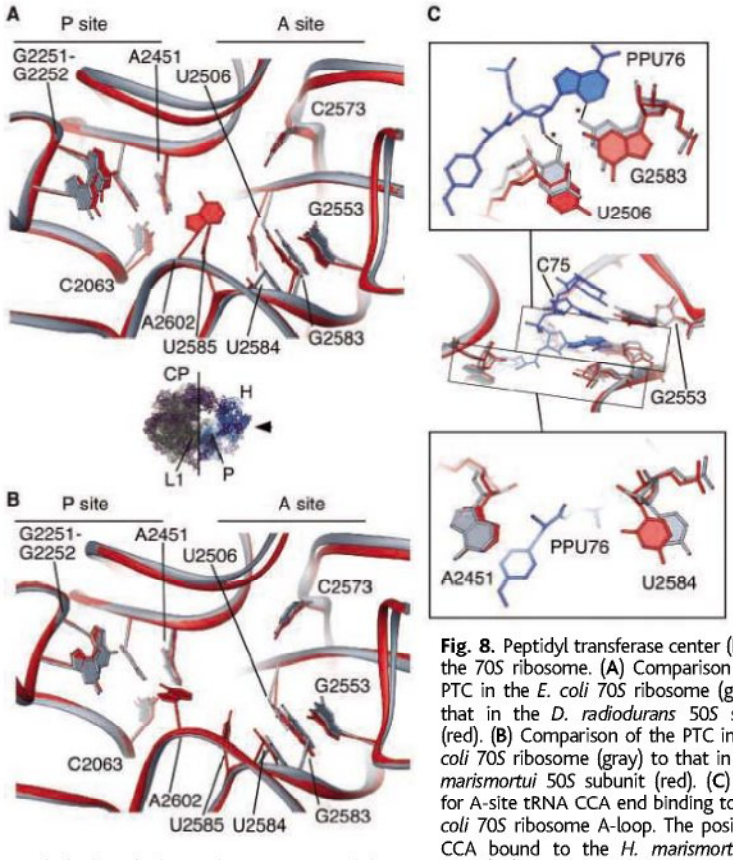

Fig. 8. Peptidyl transferase center (PTC) in the 70*S* ribosome. (**A**) Comparison of the PTC in the *E. coli* 70*S* ribosome (gray) to that in the *D. radiodurans* 50*S* subunit (red). (**B**) Comparison of the PTC in the *E. coli* 70*S* ribosome (gray) to that in the *H. marismortui* 50*S* subunit (red). (**C**) Model for A-site tRNA CCA end binding to the *E. coli* 70*S* ribosome A-loop. The position of CCA bound to the *H. marismortui* 50*S* subunit was docked on the basis of superposition of the entire PTC (*33*). Asterisks indicate minor-groove interactions of ~2 Å, if A76 is not shifted to account for the shifts in the *E. coli* rRNA.

the two ribosome structures, where electron density for the base is missing in simulated annealing omit electron density maps for ribosome I (fig. S8). This base is flipped out of the base pair, with G2583 toward U2585 in one *H. marismortui* 50*S* subunit structure with bound CCA trinucleotide substrate analogs (*5*), and is not paired in the *D. radiodurans* structure (*11*). Furthermore, two critical PTC nucleotides in the innermost layer surrounding the CCA ends of the tRNA substrates (*3*, *69*) are disordered in the 70*S* ribosome (bases of U2585 and A2602; fig. S8). These nucleotides adopt different conformations depending on the nature of the substrates bound in the 50*S* subunit structures (*3*, *5*, *70*, *73*). The degree of flexibility seen in the 70*S* ribosome PTC, along with a more "open" conformation, may contribute to more rapid peptide bond formation in the 70*S* ribosome relative to the 50*S* subunit.

Conclusion. Comparisons of the two structures of the *E. coli* ribosome to previously determined x-ray crystal structures and cryo-EM reconstructions indicate that the EF-G catalyzed unlocking event during translocation (*30*, *31*) involves opening of the G1338-U1341 ridge between the P and E sites on the small subunit. This model can now be tested. The degree to which large-scale motions in the ribosome are concentrated in RNA helices is surprising, given the universal conservation of nearby residues (*34*). High-resolution structures of the ribosome in the different functional states that drive these large-scale movements will be needed to determine how they are controlled. Also noteworthy is the high level of solvation at the subunit interface, especially in the center of the small-subunit platform and in bridge B6 (*12*). The functional roles of such an interface and how the interface rearranges during translocation remain to be determined.

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REPORTS

The Onset of Planet Formation in Brown Dwarf Disks

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The onset of planet formation in protoplanetary disks is marked by the growth and crystallization of sub–micrometer-sized dust grains accompanied by dust settling toward the disk mid-plane. Here, we present infrared spectra of disks around brown dwarfs and brown dwarf candidates. We show that all three processes occur in such cool disks in a way similar or identical to that in disks around low- and intermediate-mass stars. These results indicate that the onset of planet formation extends to disks around brown dwarfs, suggesting that planet formation is a robust process occurring in most young circumstellar disks.

Planet formation starts with the growth of submicrometer-sized amorphous grains in the protoplanetary disks [e.g., (*1*, *2*)]. Theory predicts that larger grains settle faster to the disk mid-plane, resulting in flattened disk geometries [e.g., (*2*, *3*)]. Observational evidence for dust settling has been found for disks around young, low-mass (T Tauri) and intermediate-mass (Herbig Ae/Be) stars [e.g., (*4*)]. Substantial dust processing in the early solar system is demonstrated by the high crystallinity of some comets containing dust from the epoch of their formation (*5*). Recently, intermediate-mass stars were shown to have high crystallinity only when larger grains are present (*6*), suggesting a possible link between grain growth and crystallization. Up to now, detailed dust composition studies were limited to bright disks of intermediate-mass stars, which suggested very low or no crystallinity for disks around low-mass stars [e.g., (*6*)]. If true, dust processing would strongly depend on the stellar properties, and planet formation processes would differ or not occur at all in disks of very-low-mass stars. Recently, accumulating evidence indicates that crystalline silicates are present in disks of low-mass stars [e.g., (*7*, *8*)]. Even more surprisingly, ground-based photometry of a brown dwarf disk showed hints for grain growth and dust settling (*9*), and crystalline silicate features were identified in the disk of a brown dwarf candidate (*10*). These findings set the question of whether such few Jupiter-mass disks (*11*, *12*) can form planets. In this report, we present mid-infrared spectra of disks around very-low-mass young stellar and substellar objects. We show that five out of six disks have highly processed dust: Large grains and very high crystalline mass fractions (~40%) are found. The correlation between the shape and strength of the silicate emission feature observed for Herbig Ae/Be disks extends to brown dwarf disks, demonstrating that dust processing is independent of the stellar properties. All the disks with highly processed dust have strongly flattened disk structure, as expected from dust settling. We conclude that the first steps of planet formation occurred in these brown dwarf disks, suggesting that even substellar disks can form planets.

We used the Spitzer Space Telescope and its sensitive Infrared Spectrograph to survey the complete population of substellar-mass objects with previously identified mid-infrared excess emission (*13*, *14*) in the Chamaeleon I star-forming region. We obtained low-resolution ($\lambda/\Delta\lambda \sim 60$ to 120) infrared spectra between 7.7 and 14.4 μm, covering the 10-μm silicate emission feature, whose shape and strength is determined by dust grain size and composition. Our targets have been spectroscopically classified as brown dwarf candidates or objects on the stellar-substellar boundary (*13*–*16*). The observations provide a yet-unique coeval sample (1 to 3 My) of cool objects with temperatures between 2500 and 3100 K, expanding the range in stellar mass over which dust composition has been studied to two orders of magnitude, a factor of two in temperature, and four orders of magnitude in luminosity. The spectra were taken by using multiple ramp cycles and reduced with the Spectroscopic Modeling, Analysis, and Reduction Tool (SMART) reduction package and routines we developed (*16*, *17*). We confirm mid-infrared excess emission, indicative of disks, for six of our targets; the fluxes of the two other objects (Cha 449 and Cha 425) are consistent with pure photospheric emission and are excluded from further analysis.

The spectra allow morphological comparison with the infrared spectrum of the interstellar medium and that of comet Hale-Bopp, as shown in Fig. 1. All six brown dwarf disks have emission features substantially broader than that of the interstellar medium, indicative of larger grains [e.g., (*18*)]. Whereas the spectrum of Cha Hα1 peaks at 9.8 μm, similarly to the dominantly amorphous interstellar grains, the other five targets show prominent crystalline silicate emission features with characteristic peaks at 9.3 μm and 11.3 μm. In particular, the spectrum of Cha 410 resembles that of comet Hale-Bopp. The faintest of our targets, Cha Hα9, shows a strong crystalline contribution, but because of its low signal-to-noise ratio we excluded it from the further quantitative analysis. To link grain growth and crystallization (*7*, *19*), we studied the relationship between the strength and the shape of the silicate feature by plotting the flux ratio at 11.3 μm and 9.8 μm against the peak over continuum flux ratio (Fig. 2). To ensure homogeneity, we used a single procedure to derive these values for young intermediate-mass stars (*6*), low-mass stars (*7*), and our brown dwarf sample. For intermediate-mass stars, a linear correlation has been proposed by (*6*): Weaker features have more crystalline contribution. We show that this correlation is also valid to disks around low-mass stars as well as for brown

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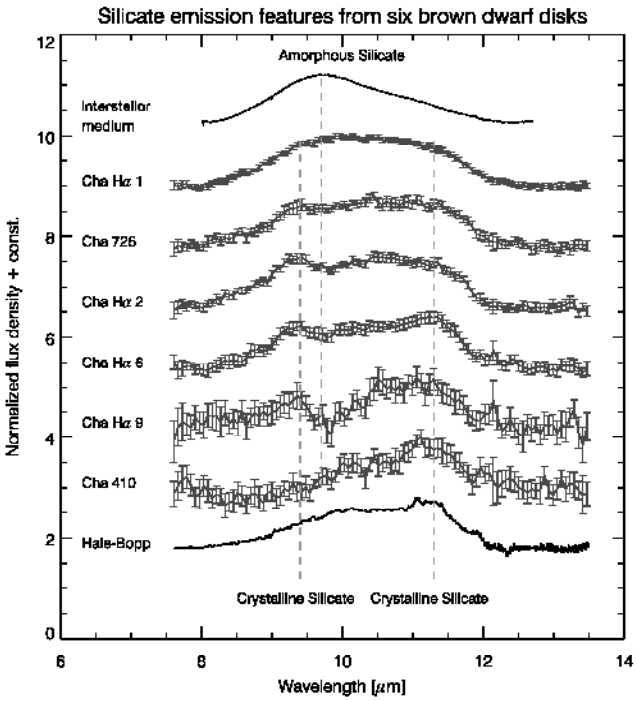

Fig. 1. Continuum-subtracted and normalized silicate emission features from our targets. For comparison, the spectra of the amorphous silicate-dominated interstellar medium and the crystalline-rich comet Hale-Bopp (*5*) are also shown. The 9.3-μm peak is mainly enstatite; the 11.3-μm peak is from forsterite. const., constant.

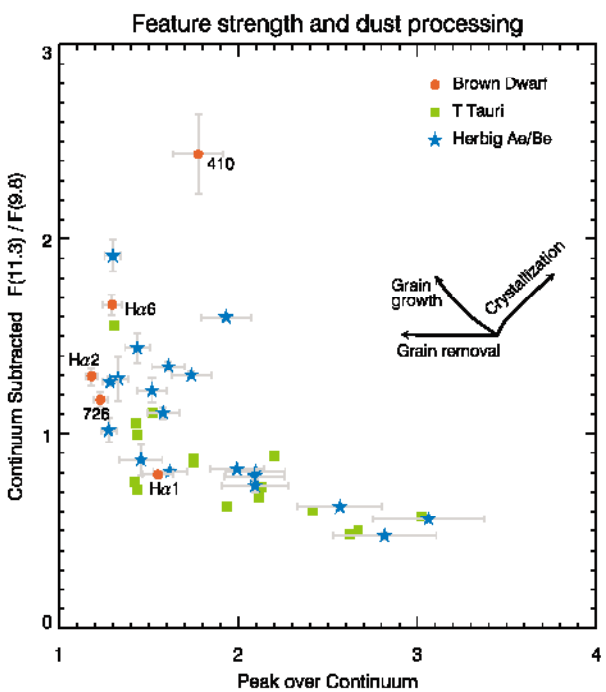

Fig. 2. Crystalline contribution to the silicate feature [flux at 11.3 μm [F(11.3)] over flux at 9.8 μm [F(9.8)]] as a function of the emission feature strength (peak flux over continuum flux). The correlation recognized for intermediate- and low-mass young stars (Herbig Ae and T Tauri) holds for brown dwarfs, but it is not linear.

dwarf disks. On the basis of a simulation of a silicate emission feature and alterations of its dust composition, we also plot three vectors indicating small grain removal, grain growth, and grain crystallization. The locations of our targets in the plot demonstrate that dust processing (grain growth, grain removal, and/or crystallization) occurred in all of them. The fact that the disks around intermediate-, low-, and substellar mass objects follow the same correlation shows that the dust processing is very similar or identical in these systems. Because of the nature of the plotted quantities, the correlation becomes nonlinear for highly processed dust (flux at 11.3 μm is greater than or equal to flux at 9.8 μm) when the crystalline emission feature becomes dominant. We suggest that for very strongly processed grains the feature strength may increase with increasing crystallinity and will reverse the observed correlation. The plot demonstrates that high crystallinity is always accompanied by very similar grain growth for stars of all masses, suggesting a direct link between these processes and very similar time scales. The reliable age estimate of our clustered targets proves that substantial dust processing occurs as rapidly as 1 to 3 My. Such rapid dust processing argues either for efficient radial mixing, if the dust is crystallized in the inner disk (*20*), or for the early heating of the disk through accretion leading to crystallization at larger disk radii.

We quantify the dust processing by decomposing the observed spectra into emission from five dust species (*16*) (fig. S2), following the method applied for intermediate-mass stars (*6*, *18*). For comparison purposes, we opted to use the same five major dust species (amorphous: olivine, pyroxene, and silica; crystalline: forsterite and enstatite), each with two grain sizes (0.1-μm and 1.6-μm radii), to allow characterization of crystallinity and grain growth in the upper disk layer observable in the mid-infrared. The quantitative study of the five brown dwarf disks confirms the morphological comparison of the spectra: four of the sources have significant contribution from crystalline silicates (9 to 48% mass fractions) and from large grains. As a next step we plotted the crystalline mass fractions in disks of brown dwarfs of low- (*21*) and of intermediate-mass (*6*) stars against the (sub)stellar mass and temperature (Fig. 3). Four out of five disks have high crystalline mass fractions compared with the disks of intermediate- and low-mass stars. Remarkably, the previously suggested trend of crystallinity increasing with the stellar mass [predicting little or no crystallinity for low-mass stars (*6*)] is not valid. Our data show a slight increase in crystallinity with decreasing stellar temperatures. This trend can be interpreted keeping in mind that infrared observations sample smaller radii for the cooler disks than for the warmer ones. If crystals form in the inner disk and diffuse outward via turbulence in a similar way in all disks, probing the inner disks will result in higher crystalline contribution. Apart from this slight increase, no obvious, direct relation exists between the stellar temperature and crystallinity. We conclude that the crystallinity is largely independent of the stellar properties and is likely determined by local processes in the disk, such as the efficiency of radial mixing.

We find an apparent anticorrelation of crystalline mass fraction with the age of the objects, also noted by (*6*) for intermediate-mass young stars; however, the large uncertainties in the age estimates for the isolated intermediate-mass stars do not yet allow any firm conclusions (*17*).

Whereas the silicate emission feature is determined by the dust grain properties, the continuum of the spectrum is defined by the disk geometry. In Fig. 4 we plot the flux densities outside the silicate emission feature (at 8 and 13 μm) and compare them with the emission

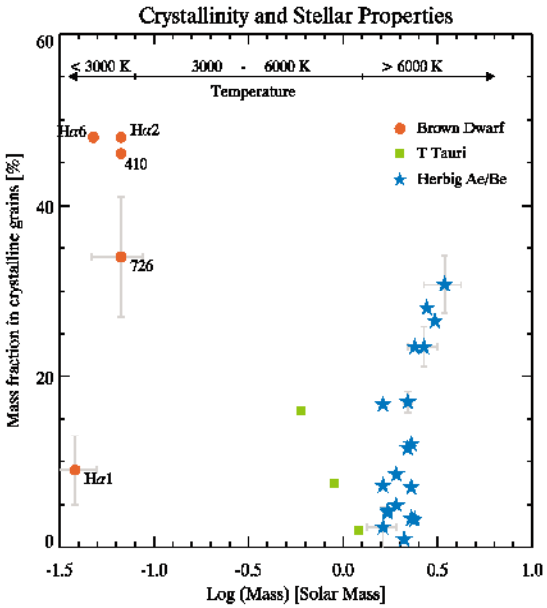

Fig. 3. Stellar and substellar mass and temperature as a function of the crystalline mass fraction. Measurements of the brown dwarf disks probe the relation over a much broader parameter range than previous observations. The suggestive decline of crystallinity with stellar mass apparent for Herbig Ae/Be stars is not a general trend: Even very low mass and cool objects can harbor highly crystallized disks. Typical uncertainties are indicated (error bars).

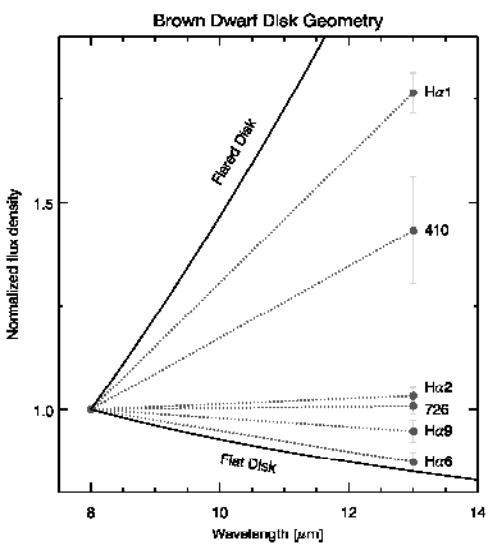

Fig. 4. Dust settling in six brown dwarf disks. The flux densities of the disks at 8 and 13 μm are normalized to the 8-μm flux and plotted against the wavelength. The slopes of the spectral energy distributions for flat and flared disks are overplotted. All disks show intermediate flaring; four disks are close to the flat geometry.

from flat and flared disk models (*22*). Disks with "flared" geometry (disk opening angle increasing with the radius) intercept more stellar light, leading to steeply increasing emission toward longer wavelengths. Maintaining the flared geometry requires turbulent gas to keep the dust from settling to the disk mid-plane. Disk models suggest that with increasing dust grain size the gas-dust coupling becomes ineffective, leading to the gradual settling of the dust toward the mid-plane and a flatter disk structure. In this evolutionary picture, all gas-rich disks start with flared geometry and will evolve to flat disks as the result of dust evolution. In agreement with this sequence, we find that the five disks with highly processed dust have intermediate or flat disk geometries, demonstrating that dust settling has occurred similarly to that in disks of low-mass stars [e.g., (*4*)]. The sixth object, Cha Hα1, is closer to the flared disk model, consistent with the less processed grains as derived in Fig. 2. The identified dust settling implies that dust grains larger than those observed in the upper, optically thin disk layer are present close to the disk mid-plane (*23*).

Here, we showed that grain growth, crystallization, and dust settling have occurred in brown dwarf disks. It appears that the first steps of planet formation are very similar or identical in disks around intermediate- and low-mass stars and even in the substellar regime. We suggest that the central star does not play a key role in the planet formation steps beyond grain growth: Large grains (>100 μm) or small planetesimals will evolve independently of the central object. It seems thus likely that planetesimals, and eventually planets, will also form in brown dwarf disks, underlining the robustness of planet formation.

We speculate on the expected planetary architectures by scaling the minimum-mass solar nebula [10 to 70 Jupiter mass (*24*)] to the mass of the two brown dwarf disks with measured masses (*12*). The minimum-mass solar nebula gives birth to an ensemble of planets with 1 Jupiter mass and below: If the brown dwarf disk mass is distributed in a similar fashion, the most massive planets are expected to be Neptune-like. There should be enough material present to form terrestrial planets in the inner disks, and therefore the closest brown dwarfs should be important targets for future planet searches.

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Supporting Online Material

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A Sporadic Third Layer in the Ionosphere of Mars

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The daytime martian ionosphere has been observed as a two-layer structure with electron densities that peak at altitudes between about 110 and 130 kilometers. The Mars Express Orbiter Radio Science Experiment on the European Mars Express spacecraft observed, in 10 out of 120 electron density profiles, a third ionospheric layer at altitude ranges of 65 to 110 kilometers, where electron densities, on average, peaked at 0.8×10^{10} per cubic meter. Such a layer has been predicted to be permanent and continuous. Its origin has been attributed to ablation of meteors and charge exchange of magnesium and iron. Our observations imply that this layer is present sporadically and locally.

Photochemical processes control the behavior of the ionospheric layers of Mars, in a manner similar to the layers of Earth or Venus. The first detection of a strong main layer of the martian ionosphere, at an altitude of 135 km, was accomplished in 1964 with Mariner 4 by use of the radio occultation method (*1*). Indications of a second, lower layer at 110 km were seen with Viking (*2*) in 1977. Mars Global Surveyor (MGS) has conducted further studies of these layers (*3*) since 1999. Although the neutral atmosphere is mainly composed of CO_2, the main ion found by the Retarding Potential Analysers (*4*) on board the Viking landers in the altitude range of both layers was O_2^+, which forms as a result of molecular reactions of oxygen with ionized carbon dioxide.

The Mars Express Orbiter Radio Science Experiment (MaRS) carried by the European Space Agency (ESA) spacecraft Mars Express (MEX) sounded the martian atmosphere and ionosphere during the first (April to mid-August 2004) and second (December 2004 to January 2005) Earth occultation seasons. MEX used a radio carrier uplink frequency of ~7100 MHz and corresponding, coherent downlink frequencies of ~2300 and 8400 MHz. During occultation events, the spacecraft disappeared behind the planetary disk of Mars as seen from the Earth, with the result that the MEX uplink and downlink radio signals passed through the ionosphere and atmosphere from the surface to a maximum altitude of ~1500 km (*5*). We report here a specific feature seen in some of the MEX ionospheric electron density profiles: the presence of a sporadic third layer between 65 and 110 km in altitude, well below the previously known two higher layers.

During the first occultation season (*6*), the MaRS experiment retrieved 90 profiles of pressure, temperature, and neutral number density from the lower atmosphere, together with profiles of electron density from the overlying ionosphere. Another 30 profiles of the neutral and ionized atmosphere were obtained during the second occultation season (*7*). The data set from the first occultation season may be divided into two parts comprising 13 profiles obtained in April 2004 [solar longitude (L_s) = 13° to 21°], covering solar zenith angles 85° to 108° at local morning times of about 04:30 to 05:15, and 77 profiles (L_s = 32° to 74°), covering solar zenith angles 70° to 84° in late afternoon at about 17:00. A stable, two-layer ionospheric structure with peak altitudes of ~130 and ~110 km for layers M1 and M2, respectively, was evident during the day (Fig. 1A) (*8*). The peak electron densities were on the order of 10^{11} electrons per cubic meter for the M1 layer and ~50 percent less for the M2 layer (*9*). Although clearly absent in Fig. 1A, a third layer (M3) can be seen in Fig. 1B, where the data has been treated in the same manner.

The third layer is evident in 10 of 120 observed MaRS profiles, as indicated by a clear increase in electron density of about 2 to 8 times one standard deviation of the electron density fluctuations. Part of the third layer is hidden in the lower portion of the second layer; this aspect of the profile is revealed by subtracting fits to M1 and M2 from each observation model (Fig. 2). In instances when the third layer was not present, the lower ionosphere is accurately represented by a superposition of two Chapman density functions (Fig. 1A). The residuals in Fig. 1 are of the magnitude of the general electron density fluctuations observed in the topside ionosphere.

The occurrence of the third layer was not limited to specific times of the day or locations. We observed this layer in the early morning and afternoon in the northern hemisphere, in the equatorial region, and at mid-southern latitudes. The layer was not found in the 20 ionospheric observations at night, all of which were at high southern latitudes during winter. The observations are too limited to exclude the occurrence

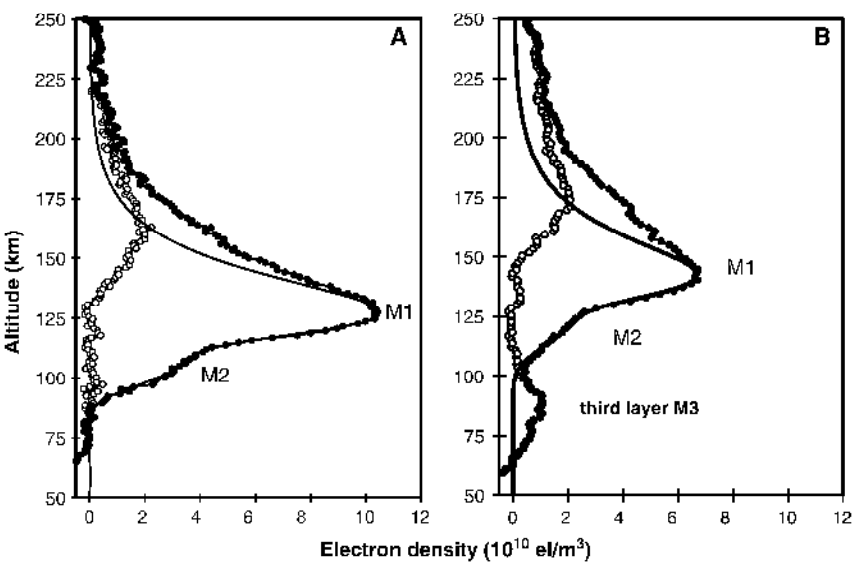

Fig. 1. (A) The typical stable two-layer structure of the electron density in the martian ionosphere (solid circles), with main layer M1 and secondary layer M2 observed on DOY 171, 2004, orbit 527, at the ESA ground station New Norcia. A Chapman ionization model fit to the lower ionospheric densities (solid line) was substracted from the observations in order to assess the magnitude of the electron density fluctuation (open circles), which is not higher than that at much higher altitudes. el, electrons. (B) Data from observations similar to those in (A) on DOY 109, orbit 314, showing layer M3 with peak ionization near 90 km. Peak density in the layer was ~8σ in the 100- to 150-km altitude range (open circles). This layer was present in ~10% of observed profiles in the 75- to 105-km altitude range. Altitude is with respect to the Mars Orbiter Laser Altimeter reference surface.

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of a third layer at night; however, it is clearly somewhat rare during the conditions of observations reported here (table S1).

A third layer containing metallic ions has been predicted to form between 80 and 100 km in altitude (*10*, *12*). The source of these ions is charge exchange of Fe and Mg derived from ablation from meteorites entering the martian atmosphere. This phenomenon is well known in Earth's ionosphere at an altitude of 95 to 100 km (*13*).

The MaRS data show that the observed layer is not a permanent and continuous feature of Mars' upper atmosphere but is geographically localized and sporadic. The third layer was detected on two consecutive days, with observations three orbits apart (orbit pairs 292, 295 and 311, 314). In both cases, the locations of the profiles were in a 3° latitude and 30° longitude range. Apparently, if the third layer is formed by meteoric ablation, the recombination rate must be low and the metallic ions must remain in the local area and altitude range for a time that is longer than the time difference of ~20.1 hours between three subsequent orbits. This corresponds to 294° of Mars rotation, in order to be consistent with these observations. Our analysis of orbits 525 to 528 provides evidence of the layer's localization in space. We observed the third layer during orbit 526, although this layer was absent in the preceding orbit 525 and in the following orbits 527 and 528, over areas separated by 98° in longitude.

The third layer could be formed within a dust layer present in the upper atmosphere, as seen by other instruments on MEX (*14*, *15*). This dust layer may also be created by the influx of meteors.

In order to test whether or not the third layer is a true feature of the atmosphere and not within the inherent general density fluctuations, we made use of the Chapman models fit to the observations (Fig. 1) (*16*). We subtracted the best-fit model from the 10 profiles that showed ionization below 100 km (Fig. 2). We then computed the standard deviation, σ_{iono}, from the differences between the fit and observation in the 100- to 130-km altitude range and compared the result with the residual between 60 and 100 km in altitude. The peak amplitude of the layer M3 was 10 σ_{iono} [v., table S1, day of year (DOY) 102]. The mean peak signal-to-noise ratio for the group was 6.3 σ_{iono}. For comparison, the mean amplitude of the density fluctuations well above the topside ionosphere in the altitude range between 600 and 670 km, mainly influenced by propagation of the radio carrier in the solar wind, is within 10% of that at the lower levels.

The altitude of layer M3 appears to be correlated with the altitude of the M1 peak, suggesting that the ionosphere and the formation of the third layer are related. The altitude of the peak ionization of M1 moves to lower altitudes with decreasing solar zenith angle, and the third layer peak follows the same trend. The altitude range for the morning profiles (Fig. 2A) was higher than for the afternoon profiles (Fig. 2B). The afternoon profiles appear to be more confined and narrower.

The martian atmosphere is thus of sufficient density between 70 and 100 km in altitude to provide the drag needed to heat and ablate meteorites. The density in the Earth's atmosphere above 95 km where the meteoritic layer has been found (*13*) is slightly higher than the neutral density of the martian atmosphere at 75 km (the lower range of the third layer) and is one order of magnitude higher than the martian atmosphere at 95 km in altitude. This would explain the similar ablation effect of meteorites on Mars and Earth, but the slightly thinner atmosphere at this level on Mars means that meteors of larger kinetic energies than on Earth are required for ablation to occur above 75 km. This would then explain the sporadic occurrence of the third layer at its observed altitude.

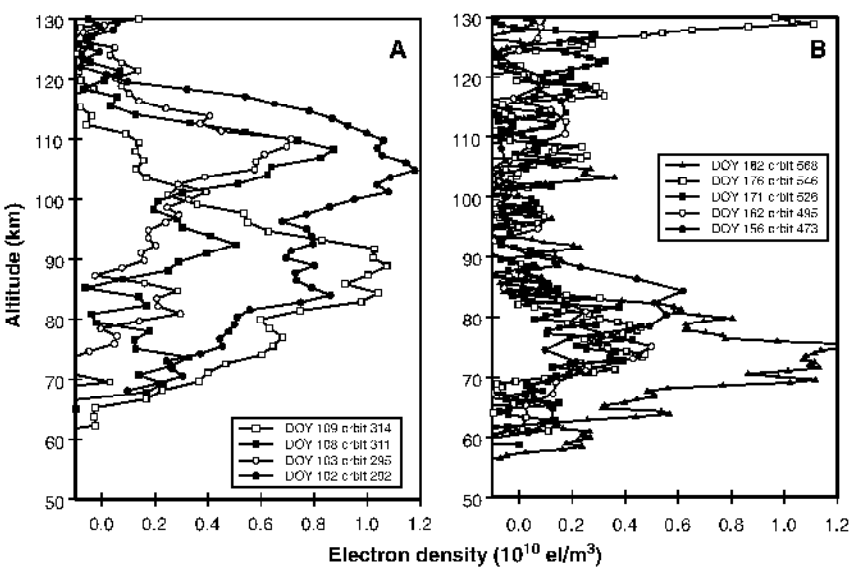

Fig. 2. Electron densities from the 10 profiles in the 50- to 130-km altitude range after removal of the Chapman profile fits to M1 and M2. In the absence of a third layer, the electron density fluctuated around 0 in the 70- to 130-km range. (**A**) Morning profiles. (**B**) Afternoon profiles.

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7. The second occultation season lasted from early December 2004 to early March 2005, but observations were terminated prematurely in early January 2005 because of power constraints on the spacecraft.
8. This notation of layers in the martian atmosphere follows Risbeth and Mendillo (*17*). MaRS had the opportunity to sound the ionosphere at the local early morning and local late afternoon. Two stable layers develop shortly before sun rise (starting at solar zenith angles of 95°) and persist into late afternoon. MGS observed a two-layer structure during other daytime hours.
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19. We thank the radio science groups in Cologne, Munich, Brussels, the Jet Propulsion Laboratory (JPL) Pasadena, and Stanford; the MEX Project Science Team at the European Space Technology Center; the Flight Control Team at the European Space Operation Center (ESOC); the ground segment groups at ESOC (New Norcia); the Deep Space Network ground stations; and S. No and T. W. Thompson at JPL for their support. The MaRS experiment is funded by the Bundesministerium für Forschung und Technologie through the Deutsches Zentrum für Luft- und Raumfahrt Bonn-Oberkassel, Germany, under grant nos. 50QM9909 and 50QM0008 and by a contract with NASA.

Supporting Online Material
www.sciencemag.org/cgi/content/full/310/5749/837/DC1
Table S1

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Meteorite Phosphates Show Constant ^{176}Lu Decay Rate Since 4557 Million Years Ago

Yuri Amelin

The use of radioactive decay of ^{176}Lu to ^{176}Hf to study the evolution of the Earth requires a precise and accurate value for the ^{176}Lu decay constant. Recent determinations of this decay constant by age comparison to the more precisely calibrated U-Pb isotopic system produced internally consistent but discrepant values between terrestrial minerals and meteorites. New highly radiogenic Lu-Hf data for phosphate minerals from Richardton (ordinary chondrite) and Acapulco (primitive achondrite) yield decay constant values of $1.864 \times 10^{-11} + 0.016 \times 10^{-11}$ and $1.832 \times 10^{-11} + 0.029 \times 10^{-11}$ year^{-1}, respectively, identical to the value determined from terrestrial minerals.

Radioactive decay of ^{176}Lu to ^{176}Hf provides a potentially powerful isotopic tracer for studying early planetary differentiation. Unfortunately, the estimate for the ^{176}Lu decay rate (λ^{176}Lu) is controversial (*1–4*).

Numerous γ-counting studies of ^{176}Lu decay [summarized in (*1*, *2*, *4–6*)] yielded a widely dispersed array of decay constant estimates. These results indicate major unresolved analytical problems in γ-counting techniques and do not constrain the λ^{176}Lu with the required precision and accuracy for geochemical applications. Age comparison studies have produced internally consistent but discrepant λ^{176}Lu values. Well-constrained Lu-Hf and U-Pb internal isochrons for terrestrial rocks younger than 2.7×10^9 years old yielded the λ^{176}Lu values of $1.865 \times 10^{-11} + 0.015 \times 10^{-11}$ year^{-1} (*7*) and $1.867 \times 10^{-11} \pm 0.008 \times 10^{-11}$ year^{-1} (*2*), whereas several Lu-Hf whole-rock isochrons for chondrites and achondrites (*8–10*) yielded values in the range from 1.93×10^{-11} to 1.98×10^{-11} year^{-1}.

Decay constants determined by age comparison are reliable only if "the initial event starting the radioisotopic clock was so short and simple as to be truly 'point-like' in time, and whose subsequent perturbations were totally nonexistent" (*1*). It is also required that the abundance of the radiogenic isotope (expressed as ^{176}Hf/^{177}Hf ratio) be sufficiently high as to make any possible initial variations insignificant. The samples used in the terrestrial age comparisons (*2*, *7*) satisfy these criteria.

Meteorites analyzed for Lu-Hf so far (*8–10*) are not so well suited for age comparison determinations of λ^{176}Lu. Chondrites contain components of variable ages: chondrules, refractory inclusions, matrix, and metamorphic minerals. Their timing of accretion, and the nature and timing of the event that caused most Lu-Hf fractionation, are uncertain. Chondrites and basaltic eucrites have relatively small variations in ^{176}Hf/^{177}Hf ratio and are therefore sensitive to a real or an apparent heterogeneity in the initial ^{176}Hf/^{177}Hf ratio [Supporting Online Material (SOM) Text]. Cumulate eucrites, which show larger variations in Lu/Hf and ^{176}Hf/^{177}Hf ratios, are metamorphosed rocks with complex and prolonged geological histories possibly spanning as much as 100 to 150 million years (My) after solar system formation [e.g., (*11*)].

Here, I report Lu-Hf and U-Pb analyses of phosphate minerals from two meteorites, primitive achondrite Acapulco and ordinary chondrite (H5) Richardton, and discuss their implications for the ^{176}Lu decay rate. Acapulco and Richardton are well suited for an age comparison study. These meteorites are recovered from observed falls and have low degrees of shock and weathering (*12–14*). More importantly, both meteorites have well-documented fast cooling histories. The cooling rate of Acapulco is higher than 1000°C/My in the temperature range above 400°C. Rapid cooling continued down to 120°C, as indicated by an (U-Th)/He age of 4538 ± 32 My (1σ), indistinguishable from the ^{207}Pb/^{206}Pb age (*15*) on Acapulco phosphates [(*16*) and references therein]. Fast cooling makes age comparison using Acapulco insensitive to the possible difference in closure temperatures between the Lu-Hf and U-Pb systems in Ca-phosphates (*17*). The average cooling rate of Richardton was estimated to be 26° – 13°C/My from 800° to 450°C (*18*) on the basis of U-Pb dates of chondrules and Ca-phosphates and experimental data for Pb diffusion in pyroxenes and apatite. This cooling rate is slower than that of Acapulco, but its influence on age comparison is still small: Even a large difference of 300°C in the closure temperature between the Lu-Hf and U-Pb systems in phosphates adds only a 12-My uncertainty to the age, corresponding to a 0.26% uncertainty in λ^{176}Lu. Phosphates from both meteorites were previously dated with the U-Pb method, which yielded ^{207}Pb/^{206}Pb ages of 4557 ± 2 My for Acapulco (*15*) and 4550.7 ± 2.6 My for Richardton (*18*). The effect of uncertainty in these ages on λ^{176}Lu determined by age comparison is insignificant. Phosphates from both meteorites have concordant (or nearly concordant) U-Pb systems, which suggests that the isotopic systems of these minerals remained closed.

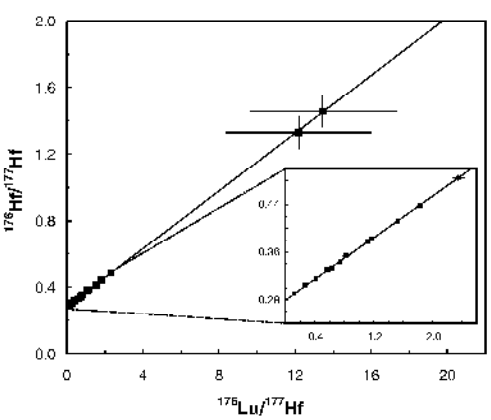

Fig. 1. Lu-Hf isochron diagram for Acapulco phosphates. Regression of all 15 Lu-Hf analyses yield an isochron with the slope of 0.08706 ± 0.0014 and the *y* intercept of 0.2802 ± 0.0012 [2σ, mean square of deviates weighted (MSWD) = 1.3, probability of fit = 0.21] (SOM Text). Error bars are 2σ, and are smaller than the plotting symbols for most analyses. The errors of ^{176}Lu/^{177}Hf and ^{176}Hf/^{177}Hf ratios are assumed to be uncorrelated, as usually done in Lu-Hf isochron calculations. However, the errors can be correlated for the fractions 2 and 6, where blank correction on ^{177}Hf is significant. Assigning error correlations of 0.9 to these fractions does not noticeably change the slope of the isochron.

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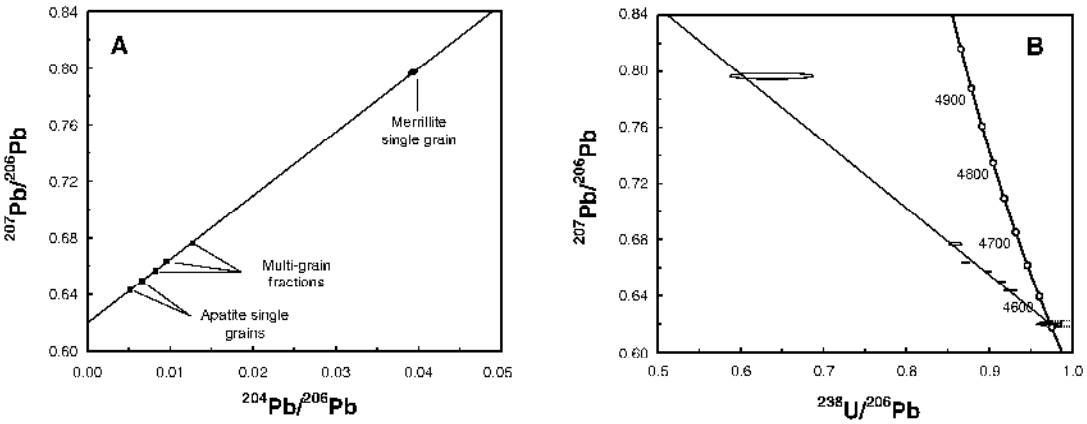

Fig. 2. (A) Pb-Pb isochron for Acapulco phosphate fractions 1 to 6. $^{207}Pb/^{206}Pb$ ratios are not corrected for initial common Pb. Isochron regression yielded an age of 4556.5 1.3 My, MSWD – 0.47, and probability of fit – 0.76. Error ellipses for apatite and multigrain analyses are smaller than the plotting symbols; error ellipse for the merrillite analysis is 2σ. (B) U-Pb three-dimensional linear regression for Acapulco phosphate fractions 1 to 6. The $^{207}Pb/^{206}Pb$ and $^{238}U/^{206}Pb$ ratios are not corrected for initial common Pb. The regression yielded an age of 4556.4 ± 3.2 My, MSWD – 5.0, and probability of fit – 0. Common-Pb plane intercepts at $^{206}Pb/^{204}Pb$ – 9.79 0.57 and $^{207}Pb/^{204}Pb$ – 10.60 1 0.39 give an estimate for initial Pb isotopic composition in the phosphates. Error ellipses for the U-Pb data are shown with solid lines; concordia plane projections, with dashed lines.

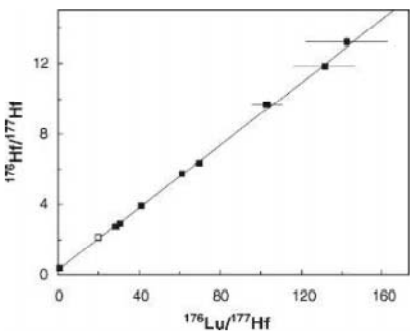

Fig. 3. Lu-Hf isochron diagram for Richardton phosphates. Error bars are 2σ and are smaller than the plotting symbols for analyses with lower $^{176}Lu/^{177}Hf$ ratios. Regression of all 10 phosphate analyses yielded an isochron with the slope of 0.08914 1 0.0023 and the *y* intercept of 0.292 0.062 (MSWD – 2.5 and probability of fit – 0.011). Exclusion of fraction 17 (shown with open symbol), which contains turbid grains, gives an isochron with a slope of 0.08855 1 0.00074 and a *y* intercept of 0.2792 0.0019 (MSWD – 0.55 and probability of fit – 0.80). The errors of $^{176}Lu/^{177}Hf$ and $^{176}Hf/^{177}Hf$ ratios are assumed to be uncorrelated, as usually done in Lu-Hf isochron calculations. The errors can be correlated for the fractions 20, 22, and 23, where blank correction on ^{177}Hf is significant. Assigning error correlations of 0.9 to these fractions changes the slope of the isochron to 0.08859 = 0.00074 and MSWD – 0.62. The difference in the slopes of the isochrons calculated with uncorrelated and correlated errors is insignificant.

Acapulco phosphates were separated (*19*) from a ~0.4-g specimen provided by K. Marti (Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, California 92093, USA). Fifteen phosphate fractions, including two single grains of apatite, one single grain of merrillite (SOM Text), and 12 multigrain fractions containing both of these minerals (*20*), were analyzed for Lu-Hf (*19*). Regression of all 15 Lu-Hf analyses produced an isochron (Fig. 1) with the slope of 0.08706 + 0.0014. Six of these fractions were also analyzed for U-Pb (*21*) and yielded a $^{207}Pb/^{206}Pb$–$^{204}Pb/^{206}Pb$ (Pb-Pb) isochron age of 4556.5 _ 1.3 My (Fig. 2A) and a three-dimensional total Pb/U isochron age (*23*) of 4556.4 _ 3.2 My (Fig. 2B). Assuming that the slope of the Lu-Hf isochron corresponds to the age of 4556.5 ⊥ 1.3 My, the decay constant of ^{176}Lu is estimated at 1.832×10^{-11} _ 0.029×10^{-11} $year^{-1}$ [with the use of the currently accepted decay constant values for U isotopes (*24*)]. The error of $\lambda^{176}Lu$ includes uncertainties in the Lu-Hf isochron slope, Pb-Pb isochron age, and the additional uncertainty of ~9.2 My from the errors of decay constants of U isotopes (*24*, *25*).

Richardton phosphates analyzed in this study are splits from the material previously analyzed for U-Pb (*18*). Ten phosphate fractions analyzed for Lu-Hf yielded an isochron with the slope of 0.08914 + 0.0023 (Fig. 3). Exclusion of fraction 17, which contains turbid grains, gives an isochron with a slope of 0.08855 _ 0.00074. This slope, combined with the Pb-Pb isochron age of Richardton phosphates of 4550.7 ⊥ 2.6 My (*18*), gives the $\lambda^{176}Lu$ estimate of 1.864×10^{-11} + 0.016×10^{-11} $year^{-1}$ (uncertainty includes all sources of errors as above).

The content of radiogenic ^{176}Hf is between 2.8 and 80.6% of the total amount of ^{176}Hf in the Acapulco phosphates and between 19.6 and 97.9% in the Richardton phosphates (table S1), therefore uncertainty and possible variations of initial $^{176}Hf/^{177}Hf$ do not significantly affect the isochrons. The *y* intercepts of both Lu-Hf isochrons (0.2802 _ 0.0012 for Acapulco and 0.2792 + 0.0019 for Richardton) agree within error with the solar system initial values obtained from whole-rock isochrons for eucrites [0.27966 ⊥ 0.00002 (*9*)] and chondrites [0.279628 – 0.000047 (*10*)], further supporting the validity of the phosphate isochrons.

A constant decay rate of ^{176}Lu over the past 4.56 Gy has several implications. First, the initial Hf isotopic ratios of rocks and minerals of any age, including the oldest zircons, should be calculated by using the decay constant value determined from terrestrial minerals (*2*, *7*). Second, the reliably known decay constant is a precondition for determination of the initial state of the Lu-Hf system in the Earth and the solar system.

Extreme fractionation of Lu and Hf by meteoritic phosphates (the highest measured $^{176}Lu/^{177}Hf$ ratio of 142 in Richardton fraction 22 is about 4000 times higher than the average chondritic value) places severe limits on the applicability of chondrites for determination of the solar system initial $^{176}Lu/^{177}Hf$ ratio. Equilibrated ordinary chondrites, in which a substantial portion of Lu is contained in Ca phosphates (SOM Text), are especially prone to phosphate-related Lu-Hf fractionation. Considering the degree of Lu-Hf fractionation in phosphates and association of phosphate grains with metal [(*26*) and references therein], it is unlikely that even the most careful crushing of equilibrated ordinary chondrites can produce powders with homogeneous Lu/Hf ratio. The most accessible group of carbonaceous chondrites, the CV chondrites, contain abundant refractory Ca-Al-rich inclusions with strong volatility-related fractionation of rare earth elements (*27*) and therefore possibly fractionated Lu/Hf. Primitive chondrites, which were not affected by a high-temperature metamorphism, have ratios of major refractory lithophile elements close to the solar photosphere values (*28*): CI, possibly CM, CR, and most primitive ordinary chondrites might be better candidates for determination of the initial solar system $^{176}Lu/^{177}Hf$ ratio.

The evidence that the spread of Lu/Hf ratios is controlled by phosphate abundance (SOM Text) is hard to reconcile with the model of

enhancement of the ^{176}Lu decay by gamma-induced photoexcitation (*29*). Most chondritic phosphates have ages similar to or younger than the Richardton and Acapulco phosphates analyzed here (*26*) and thus postdate the proposed photoexcitation. In this case, the slope of a whole rock chondritic Lu-IIf isochron is expected to be similar to the slopes of meteoritic phosphate isochrons.

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30. I thank K. Marti for the sample of Acapulco, the Mineralogy Department of the Royal Ontario Museum for the sample of Richardton, F. Corfu and M. Bizzarro for aliquots of their Lu-Hf reference solutions, T. Pestaj and J. Peressini for help with mineral separations, and L. Cataldo for help with cleaning the sample introduction system and with maintaining the good working order of the Nu Plasma (Nu Instruments, Wrexham, UK). Comments by D. Davis, B. Davis, F. Albarède, and two journal reviewers improved the paper.

Supporting Online Material

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Materials and Methods
SOM Text
Fig. S1
Tables S1 to S3
References and Notes

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The Radiative Signature of Upper Tropospheric Moistening

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Climate models predict that the concentration of water vapor in the upper troposphere could double by the end of the century as a result of increases in greenhouse gases. Such moistening plays a key role in amplifying the rate at which the climate warms in response to anthropogenic activities, but has been difficult to detect because of deficiencies in conventional observing systems. We use satellite measurements to highlight a distinct radiative signature of upper tropospheric moistening over the period 1982 to 2004. The observed moistening is accurately captured by climate model simulations and lends further credence to model projections of future global warming.

The importance of water vapor in regulating climate is undisputed. It is the dominant greenhouse gas, trapping more of Earth's heat than any other gaseous constituent (*1*). As the climate warms in response to increases in other greenhouse gases such as carbon dioxide, the concentrations of water vapor are expected to increase (*2*–*7*). If water vapor concentrations do increase in a warmer world, the added absorption will act to further amplify the initial warming. Models of Earth's climate suggest that this serves as a powerful positive feedback, more than doubling the sensitivity of the surface temperature to an anthropogenic forcing (*8*–*11*).

All climate models predict that the concentration of water vapor in the upper troposphere will increase markedly in the future (*9*, *12*). However, the validity of such projections has been debated for more than a decade (*13*, *14*). Some argue that the concentrations in the upper troposphere might actually decrease in a warmer climate, given the simplified treatment of convection and cloud-related processes in current models and the important role that they play in governing the distribution of moisture (*15*–*17*).

Here, we use climate model simulations and satellite measurements to demonstrate the presence of a distinct radiative signature of upper tropospheric moistening on interannual to decadal time scales. The observed moistening is consistent with model simulations and corresponds approximately to a constant relative humidity increase in upper tropospheric moisture (*18*). We further demonstrate that without such an increase, the model would be unable to reproduce the satellite-observed radiance record.

The distribution of water vapor is highly variable in both space and time. Because the equilibrium vapor pressure of water depends strongly on temperature, the concentration of water vapor diminishes rapidly with height. Yet because the absorptivity of water vapor is proportional to the logarithm of its concentration, it is the fractional change in water vapor mass, not the absolute change, that governs its strength as a feedback mechanism.

Model calculations of the fractional change in global mean water vapor mixing ratio (*19*) using the Geophysical Fluid Dynamics Laboratory (GFDL) atmospheric general circulation model (GCM) (*20*) indicate a distinct upper tropospheric amplification to the simulated changes in water vapor over the past two decades (*21*) (fig. S1). A similar vertical amplification is predicted to occur over the coming century in response to increases in anthropogenic greenhouse gases and is a robust feature of all climate model projections (*9*, *12*, *18*). Simulations of the 21st-century climate from the GFDL coupled ocean-atmosphere model indicate an increase in lower tropospheric water vapor of ~20% by the end of the century, versus an upper tropospheric increase of ~100% (*21*) (fig. S1). Such a difference highlights the importance of upper tropospheric water vapor as a feedback mechanism, and of its measurement as a factor in the detection and attribution of climate change.

Previous studies have demonstrated the presence of regional moistening trends in the lower troposphere since the mid-1970s from radiosonde measurements (*22*, *23*). Increases in the total column water vapor mass (*19*) have

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also been observed over the global oceans since the mid-1980s from satellites (*24*, *25*). These lower tropospheric moistening trends are strongly linked to changes in surface temperature and are consistent with those expected under the assumption of constant relative humidity.

The model used here is an atmospheric GCM integrated with specified sea surface temperatures (SSTs) (*20*). Four sets of model integrations are performed, each starting in January 1982 when satellite-observed SSTs became available, and ending in December 2004. Because the global anomalies of the ensemble members are nearly identical, only the results from the first member are displayed in the figures, but the trends and standard errors of the trends for all members are summarized in Table 1 (*26*).

When forced with observed SSTs, this model successfully reproduces the observed column-integrated moistening changes over this period (Fig. 1). However, because the mass of water vapor decreases rapidly with height, the column integral is primarily weighted by the lower troposphere, and its largely thermodynamic behavior is unsurprising (*21*). Consequently, there is not much debate about the projected increase of column-integrated water vapor in response to global warming, and its agreement with models provides only limited reassurance in their simulation of water vapor feedback (*9*).

In contrast, water vapor in the free troposphere is not so directly constrained by thermodynamic arguments (*21*), and its response to global warming has been the subject of long-standing controversy (*9*, *15*–*17*). Given the radiative importance of moisture changes in the upper troposphere (*9*, *10*), it is important that humidity changes there are demonstrably consistent between models and observations. Although an international network of weather balloons has carried water vapor sensors for more than half a century, changes in instrumentation and poor calibration make such sensors unsuitable for detecting trends in upper tropospheric water vapor (*27*). Similarly, global reanalysis products also suffer from spurious variability and trends related to changes in data quality and data coverage (*24*).

Satellite observations using the High Resolution Infrared Radiometer Sounder (HIRS) provide a global, temporally coherent archive of radiance measurements in the 6.3-μm water vapor absorption band from 1979 to the present. The radiance channel centered at 6.7 μm (channel 12) is sensitive to water vapor integrated over a broad layer of the upper troposphere (200 to 500 hPa) and has been widely used for studies of upper tropospheric water vapor (*28*). Because clouds strongly attenuate the infrared radiation, we restrict our analysis to clear-sky radiances in which the upwelling radiation in channel 12 is not affected by clouds (*29*).

Table 1. The linear least-squares trend (±2 standard errors of the linear trend) for satellite observations and climate model simulations of total column water vapor, T12, T2, and T2 − T12. The standard errors are estimated following Weatherhead *et al.* (*42*). The rightmost column shows the trends for which the GCM-simulated T12 was computed under the "no moistening" scenario (*21*). Satellite observed trends are computed using the SSMI total column water vapor (*25*), HIRS T12 (*21*), and MSU T2 for both RSS (*38*) and UAH (*37*). GCM trends are shown for each of the four model ensemble members. Asterisks denote results shown in Figs. 1 and 2.

Variable	Satellite	GCM	GCM (no moistening)
Total column vapor (%/decade)	1.40 ± 0.78 (SSMI)	1.20 ± 0.98*	
		1.37 ± 0.78	
		1.20 ± 0.78	
		1.32 ± 0.98	
T12 (K/decade)	0.00 ± 0.04 (HIRS)	0.06 ± 0.04*	0.24 ± 0.12*
		0.07 ± 0.04	0.24 ± 0.12
		0.08 ± 0.04	0.26 ± 0.10
		0.06 ± 0.04	0.23 ± 0.12
T2 (K/decade)	0.17 ± 0.08 (RSS)	0.19 ± 0.08*	
	0.08 ± 0.08 (UAH)	0.19 ± 0.08	
		0.21 ± 0.08	
		0.18 ± 0.08	
T2 − T12 (K/decade)	0.17 ± 0.06 (RSS)	0.13 ± 0.08*	−0.04 ± 0.04*
	0.08 ± 0.06 (UAH)	0.12 ± 0.06	−0.05 ± 0.06
		0.14 ± 0.06	−0.05 ± 0.04
		0.13 ± 0.06	−0.05 ± 0.04

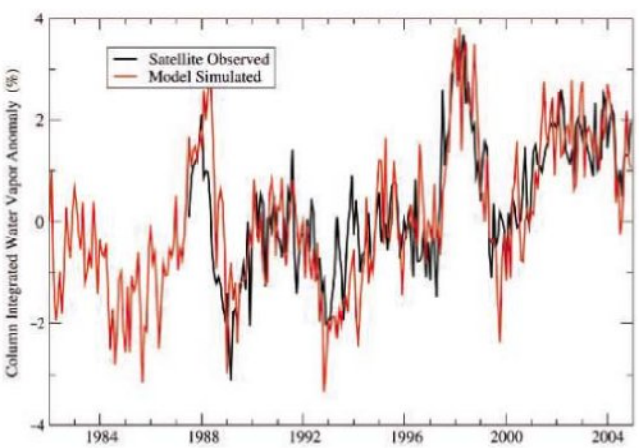

Fig. 1. Global mean (ocean-only) anomalies in column-integrated water vapor from GFDL atmospheric GCM simulations forced with observed SSTs [(*20*); red] and satellite observations from the Special Sensor Microwave Imager (SSMI) [(*25*); black].

Figure 2 compares the satellite-observed equivalent blackbody temperatures from channel 12 (T12) from the HIRS instrument with those computed from the model's temperature and moisture profiles (*30*). Under clear skies, T12 is primarily sensitive to changes in relative humidity averaged over a deep layer of the upper troposphere (roughly 200 to 500 hPa) (*21*). Thus, if the water vapor mass in the upper troposphere increases by conserving relative humidity as the atmosphere warms, only a small perturbation to T12 would be expected.

Although substantial trends in T12 do occur regionally (*31*, *32*), the globally averaged radiance record from HIRS shows little trend over the 20-year period. This lack of trend has been noted in previous studies (*21*, *33*–*36*) and is insensitive to the intercalibration of the radiance records from individual satellites (*21*). The model simulations also yield little trend in global mean T12, implying that there is little change in global mean relative humidity over this period. In fact, the model-simulated anomalies are nearly identical to those obtained if one repeats the calculation of T12 under the assumption of a constant relative humidity change in the model's water vapor field (*21*). This confirms that both the observations and GCM simulations are, to first order, consistent with a constant relative humidity behavior.

In contrast, consider the trend in T12 that would result if there were no increase in water vapor mass in the model's upper troposphere (Fig. 2, top panel, green curve) (*21*). In this case, the T12 would increase by more than 0.5 K over the period from 1982 to 2000, more than four times what was observed, as the result of an increase in Planck emission (warming) without a compensating increase in atmospheric attenuation (moistening). This discrepancy is much larger than the standard error in the trend estimates and is consistent across all GCM ensemble members (Table 1). Thus,

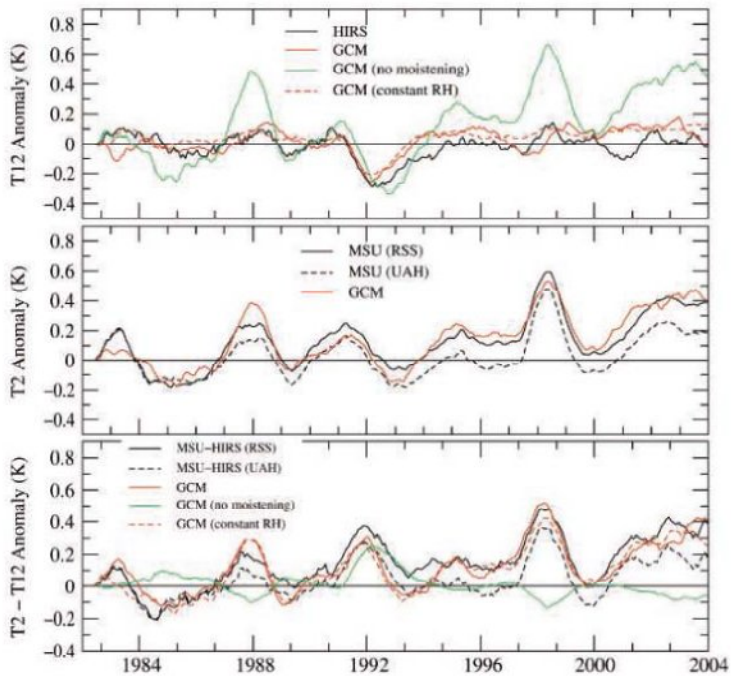

Fig. 2. Global mean time series of T12 (top), T2 (middle), and T2 – T12 (bottom) from GCM simulations (red) and satellite observations (black). The model-simulated radiances are also shown from calculations using a seasonally varying climatological profile with no moistening trend [green line (*21*)] and a prescribed moisture profile that moistens at a constant relative humidity rate [red dashed line (*21*)]. All time series are smoothed with a 6-month running mean.

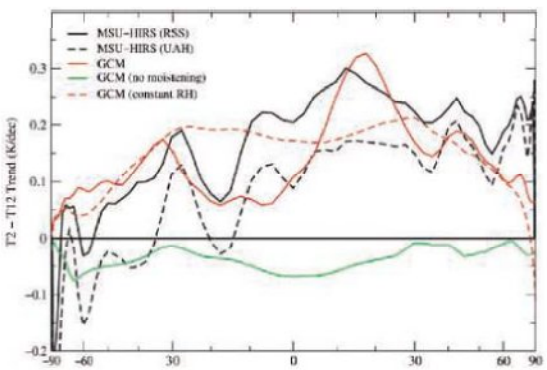

Fig. 3. An area-weighted projection of the zonal mean of the local trend in T2 – T12 (K/decade) for satellite observations (black) and GCM simulations (red). The corresponding trends in T2 – T12 for model-simulated radiances under the constraint of no moistening [green line (*21*)] and constant relative humidity moistening [red dashed line (*21*)] are also shown.

the model would be unable to reproduce the observed radiance record without a nearly constant relative-humidity moistening of the upper troposphere.

Because the anomalies in T12 are a function of both moisture and temperature changes over this period, it is important to verify the credibility of the model-simulated temperature variations. For this purpose, the global mean tropospheric temperature anomalies observed from the Microwave Sounding Unit (MSU) channel 2 radiances (T2) are compared with those simulated from the GCM (Fig. 2, middle panel). The MSU T2 radiances are primarily sensitive to the temperature averaged over a deep layer of the troposphere (roughly 200 to 800 hPa). Observations are shown for both the University of Alabama Huntsville (UAH) (*37*) and Remote Sensing Systems (RSS) (*38*) versions of MSU channel 2. Over the period 1982 to 2004, the GCM-simulated T2 anomalies are nearly identical in pattern to those observed from MSU, regardless of which record is used. The linear trend of the GCM-simulated T2 (0.18 K/decade) (*21*) is in close agreement with the RSS T2 trend (0.17 K/decade). However, both the GCM and RSS trends are greater than the UAH trend by roughly a factor of 2, reflecting differences between the UAH and RSS reconstruction methods (*38*).

The trends in upper tropospheric water vapor are more easily depicted by differencing the global mean MSU channel 2 and HIRS channel 12 radiance measurements (T2 – T12). As the atmosphere moistens, the emission level for T12 increases as a result of the increasing opacity of water vapor along the satellite line of sight. On the other hand, because the concentration of oxygen does not vary by any appreciable amount, the emission level for the MSU T2 remains constant. Therefore, if the atmosphere moistens, the brightness temperature difference T2 – T12 will increase over time because of the divergence of their emission levels. If, on the other hand, the moisture in the upper troposphere does not increase, the emission level for T12 would remain unchanged, and T2 – T12 would show little change over time.

Both the HIRS observations and GCM simulations indicate an increase in T2 – T12 over this period, reflecting the moistening of the upper troposphere (Fig. 2, lower panel). The model-simulated anomalies in T2 – T12 increase at a rate of 0.14 K/decade from 1982 to 2004. However, if the concentrations of water vapor are held constant when computing the model's T12 (green curve), the trend in T2 – T12 becomes negative (–0.04 K/decade), in stark contrast with that observed.

Both RSS (0.16) and UAH (0.08) reveal increases in T2 – T12 over this period (Fig. 2, lower panel), although the magnitude of the linear trend is roughly twice as large when using RSS T2 than when using UAH T2, reflecting the uncertainty in the rate at which the troposphere has warmed over this period (*38*, *39*). However, it is important to note that although the linear trends differ between the UAH and RSS T2 values, both show similar variability at higher frequencies, and this variability is consistent with a moist-adiabatic warming (*40*) and constant relative humidity moistening of the upper troposphere. In contrast, the "no moistening" version of the model radiance simulations is unable to capture either the observed variability or the linear trend in T2 – T12.

The zonal mean of the trend in T2 – T12 (Fig. 3) further highlights the consistency between the observed and GCM-simulated radiance records, with both showing the largest increases in upper tropospheric water vapor in the tropics and smaller decreases near the poles, including a local maximum in the northern subtropics between 0° and 30°N. In contrast, the zonal mean trends in T2 – T12 for the "no moistening" scenario are negative in most latitudes, in stark contrast to either the HIRS/RSS or HIRS/UAH records. Note that there are regions where the model-simulated trend of T2 – T12 departs substantially from the constant relative humidity approximation, the cause of which warrants further scrutiny.

Upper tropospheric water vapor provides a powerful feedback for amplifying climate change, and its increase is a crucial ingredient to model projections of future global warming. An accurate understanding of the changes in upper tropospheric moisture over time is necessary to verify its role in amplifying climate

sensitivity. Reproduction of the observed radiance record requires a global moistening of the upper troposphere in response to atmospheric warming that is roughly equivalent in magnitude to that predicted under the assumption of constant relative humidity. This behavior is consistent with that simulated from current models and provides key quantitative evidence in support of their ability to predict the climate feedback from upper tropospheric water vapor. Given the importance of water vapor feedback in determining the climatic response to anthropogenic forcings, such confirmation is essential to the use of these models for global warming projections.

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19. The water vapor mixing ratio (w) is defined as the mass of water vapor per unit mass of dry air. The relative humidity (r) is determined as the ratio of the water vapor mixing ratio to its "saturated" or equilibrium value (w_s), expressed in percent: $r = 100 \times w/w_s$. The total column water vapor (W) is defined as the vertically integrated mass of water vapor per unit area in units of kg/m^2: $W = \int w\rho\, dz$, where ρ is the density of air and z is altitude, and the integration is performed from the surface to the top of the atmosphere.
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30. To avoid uncertainties associated with the inversion of satellite-measured radiances into geophysical quantities, we input the GCM profiles of temperature and water vapor mixing ratio into a narrow-band radiative transfer model to simulate the T12 that the HIRS instrument would have observed under those conditions. The radiative transfer model used here is the HIRS Fast Forward Program (HFFP) (*44*).
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Synthesis of a Stable Compound with Fivefold Bonding Between Two Chromium(I) Centers

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Although in principle transition metals can form bonds with six shared electron pairs, only quadruply bonded compounds can be isolated as stable species at room temperature. Here we show that the reduction of $\{Cr(\mu\text{-}Cl)Ar'\}_2$ [where Ar′ indicates C_6H_3-2,6(C_6H_3-2,6-Pr^i_2)$_2$ and Pr^i indicates isopropyl] with a slight excess of potassium graphite has produced a stable compound with fivefold chromium-chromium (Cr–Cr) bonding. The very air- and moisture-sensitive dark red crystals of Ar′CrCrAr′ were isolated with greater than 40% yield. X-ray diffraction revealed a Cr–Cr bond length of 1.8351(4) angstroms (where the number in parentheses indicates the standard deviation) and a planar trans-bent core geometry. These data, the structure's temperature-independent paramagnetism, and computational studies support the sharing of five electron pairs in five bonding molecular orbitals between two $3d^5$ chromium(I) ions.

A quadruple bond between metal centers consisting of σ, 2π, and δ orbital overlaps was shown to be present in salts containing the $[Re_2Cl_8]^{2-}$ ion in 1964 (*1*). Since then, a rich chemistry has developed around this class of transition-metal compounds (*2*), whose bond order exceeds the previously known limit of three for compounds of the p-block elements. Beginning in the mid-1970s, theoretical and spectroscopic investigations of diatomic transition-metal species M_2 (where M is either Cr or Mo) trapped in inert matrices at low temperatures indicated that sextuple bonds consisting of 2σ, 2π, and 2δ overlaps (derived from valence s and d atomic orbitals) could exist between these metals (*3–14*). However, such molecules have no stable existence at room temperature and so cannot be isolated for bulk manipulation.

If ligands are used to stabilize multiply bonded metal centers, their binding reduces the

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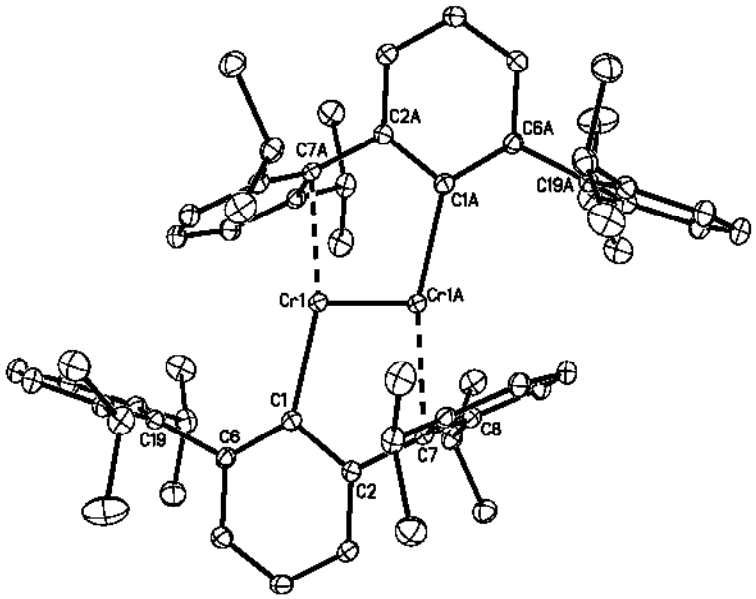


Fig. 1. Thermal ellipsoid (30%) drawing of Ar′CrCrAr′ (compound **1**). Hydrogen atoms are not shown. Selected bond distances and angles ° are as follows: Cr(1)–Cr(1A), 1.8351(4) Å; Cr(1)–C(1), 2.131(1) Å; Cr(1)–C(7A), 2.2943(9) Å; Cr(1)–C(8A), 2.479(1) Å; Cr(1)–Cr(12A), 2.414(1) Å; C(1)–C(2), 1.421(1) Å; C(1)–C(6), 1.423(2) Å; C(7)–C(8), 1.421(1) Å; C(7)–C(12), 1.424(1) Å; Cr(1A)–Cr(1)–C(1), 108.78(3)°; Cr(1A)–Cr(1)–C(7A), 94.13(3)°; C(1)–Cr(1)–C(7A), 163.00(4)°; Cr(1)–C(1)–C(2), 114.34(7)°; Cr(1)–C(1)–C(6), 131.74(7)°; and C(2)–C(1)–C(6), 113.91(9)°.

number of valence orbitals available to form metal-metal bonds. Thus, the number of ligands must be minimized, and the number of metal valence electrons that fill bonding orbitals must be maximized in order to achieve the highest bond order possible in an isolable compound. Moreover, the ligands must be sufficiently bulky to inhibit intermolecular reactions that yield clusters or polymers with lower bond orders. We have shown (*15*) that the sterically encumbering monovalent terphenyl ligand C_6H_3-2,6(C_6H_3-2,6-Pr^i_2)$_2$ (hereafter designated Ar′), where Pr^i is isopropyl, and related derivatives can stabilize many compounds with low coordination numbers or unusual bonding (*16*, *17*). We now show that this ligand allows room-temperature isolation of the Ar′CrCrAr′ chromium dimer to occur. The structural, spectroscopic, and magnetic properties of this compound are consistent with a quintuple Cr Cr bond formed by a fivefold overlap between the metal d orbitals (*18*).

The compound Ar′CrCrAr′ (compound **1**) was isolated as dark red crystals from the reduction of {Ar′Cr(μ-Cl)}$_2$ with KC_8 (*19*). The crystals are thermally robust and decompose slowly above 200°C, but they are spontaneously flammable when exposed to air. X-ray crystallography of **1** (Fig. 1) (*20*) showed a structure characterized by a center of symmetry at the midpoint of the very short [1.8351(4) Å, where the number in parentheses indicates SD] Cr–Cr bond. Each Cr is bonded to the ipso carbon atom [distance Cr(1) C(1) – 2.131(1) Å] of an Ar′ substituent. There is also a weaker interaction between each Cr ion [Cr(1) C(7A) – 2.2943(9) Å] and the ipso carbon [C(7) or C(7A)] of a flanking ring of the terphenyl group attached to the other Cr. The core atoms, C(1)Cr(1)Cr(1A)C(1A), are coplanar, but they have a trans-bent structure with C_{2h} local symmetry and a bending Cr(1A)Cr(1)C(1) angle of 102.78(3)°. Magnetic measurements revealed a temperature-independent paramagnetism of 0.000112(5) electromagnetic units (emu) per mol of Cr (*21*). The electronic absorption spectrum of **1** displays strong absorptions below 250 nm and a broad absorption at 488 nm, with an intensity (ε) of 3200 mol^{-1} L cm^{-1}.

The metal-metal bonding in compound **1** arises from the interaction of two Cr(I) centers with d^5 electron configurations. In a simplified molecular-orbital overlap diagram with the assumption of local C_{2h} symmetry, five metal-metal bonding molecular orbitals can be visualized (Fig. 2) (*22*, *23*). Also, two further metal-ligand orbital combinations, bonding and antibonding with respect to the metal-metal bond, are present. The bonding is actually more complex, because mixing of the orbitals with the same symmetry (i.e., 4s and $3d_{z^2}$ or $3d_{x^2-y^2}$) can occur. Nonetheless, σ (d_{z^2} – d_{z^2}, A_g), 2π (d_{yz} d_{yz}, d_{xz} d_{xz}, A_u, B_u), and 2δ ($d_{x^2-y^2}$ $d_{x^2-y^2}$, d_{xy} – d_{xy}, A_g, B_g) Cr–Cr overlaps, in which electrons from each metal become paired to fill the five bonding orbitals, are possible (*23*).

This fivefold Cr–Cr interaction is supported by structural and magnetic data. The Cr Cr distance is extremely short and is very close to the 1.828(2) Å bond found in the Cr(II) dimer, $Cr_2\{C_6H_3$-2-OMe-5-Me$\}_4$, which has the shortest reported metal-metal bond distance (*24*). In this Cr(II) compound and related species, the chelating nature of the ligand plays a key role in pushing the Cr centers close together, and it could be argued that the Ar′ ligand acts similarly in **1** through the secondary Cr—C interactions. However, we have also synthesized the related Ar′FeFeAr′ and Ar′CoCoAr′ dimers, which are structurally similar to **1** but have much longer Fe Fe and Co–Co distances, ~2.53 and 2.80 Å, respectively. Thus, the Ar′ ligand can accommodate M-M separations that vary by almost 1 Å. For this reason, the bridging shown by the Ar′ ligand in **1** is unlikely to be the cause of the short metal-metal distance. In other words, the very short Cr Cr bond in **1** is mainly due to the interaction of the d^5 Cr centers, rather than a constraining ligand geometry (*25*).

The temperature-independent weak paramagnetism of **1** is also consistent with strongly coupled d^5-d^5 bonding electrons. Temperature-independent paramagnetism has been observed for several other M-M–bonded transition-metal complexes (*26*–*29*). Nonetheless, the possibility that the Cr–Cr multiple bond may be a combination of covalent bonding with antiferromagnetic coupling, which was recently calculated for the Cr_2 dimer (*14*), should not be dismissed. The distinction between antiferromagnetic coupling and what constitutes a bond is not clearly defined; therefore, it would be of great interest to determine the contribution of the antiferromagnetic exchange coupling to the overall Cr Cr bond energy. This exchange coupling is so strong in **1** between 2 and 300 K that, unfortunately, there is no increase in the susceptibility as the S > 0 states are populated; i.e., $2J$, the antiferromagnetic exchange coupling, is so negative that only the S = 0 ground state is effectively populated at these temperatures. As a consequence, the susceptibility never begins to increase with increasing temperature, and it is difficult to determine $2J$. The unpopulated S > 0 excited states yield a second-order Zeeman contribution of 0.00112(5) emu/mol Cr to the molar magnetic susceptibility. This is the so-called "temperature-independent paramagnetism" (TIP), a contribution which must be added to the essentially zero contribution of the S = 0 ground state.

Further insight on the bonding in **1** may be obtained from computational data. However, calculations on multiply bonded transition-metal species have often been difficult because of electron correlation problems (*30*, *31*). Nonetheless, recent studies (*8*, *32*, *33*) have suggested that density functional theory (DFT) methods can compete successfully with high-level ab initio calculations. Both the trans-bent geometry and the quintuple-bond formulation are predicted by the simple, Lewis-like electron-

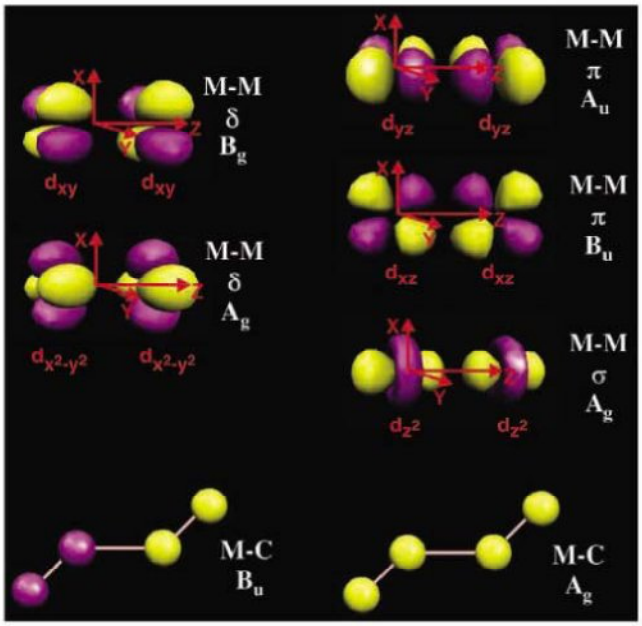
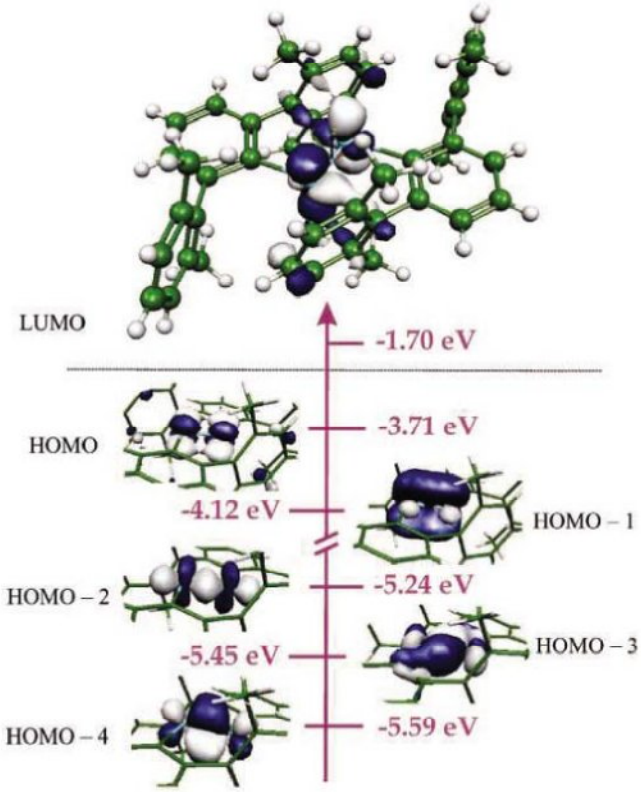


Fig. 2. (**Left**) Schematic drawing of simplified molecular orbital overlaps for M-M and M-C bonding. **Fig. 3.** (**Right**) Electron density surfaces and energies for the Cr–Cr bonding orbitals in Ar′CrCrAr′ (*36*).



pair sharing scheme of Landis and Weinhold for transition-metal complexes (*34*, *35*). We carried out restricted DFT calculations (*36*) using hybrid and pure functionals to further analyze the Cr–Cr interaction. These theoretical approaches (*37*) yielded very similar results. Molecular orbitals were generated from single-point calculations by using the atomic coordinates provided by the x-ray structure. The metal-metal orbital surfaces (Fig. 3) support the view that there are five orbital interactions between the Cr(I) ions. The symmetries of the highest occupied molecular orbital (HOMO) and HOMO − 1, which differ in energy by 0.41 eV, correspond to δ bonds. The HOMO − 2 corresponds to Cr Cr σ bonding and lies at ~1.08 eV lower energy than HOMO − 1. HOMO − 3 and HOMO − 4 correspond to Cr Cr π bonds and lie slightly (~0.21 to 0.35 eV) below the σ-bonding level.

The calculated HOMO lowest unoccupied molecular orbital (LUMO) energy gap (2.01 eV, 46.35 kcal mol^{-1}), which may correspond to a δ-δ* transition, is at a somewhat lower energy than the 58.59 kcal mol^{-1} calculated from the 488-nm absorption maximum in the electronic spectrum. This discrepancy has precedent in $\sigma^2\pi^1\delta^2$ quadruply bonded M-M species, for which the experimental δ-δ* transition energies are usually higher than those calculated (*2*). Moreover, the putative δ-δ* transition lies at the higher energy end of the ~450 to 1600-nm range observed for quadruply bonded compounds (*2*), which suggests that the δ bonds in **1** are as strong as those observed in the quadruply bonded compounds.

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18. The description "quintuple bond" is intended to indicate that five electron pairs play a role in holding the metal atoms together. It does not imply that the bond order is five or that the bonding is very strong, because the ground state of the molecule necessarily involves mixing of other higher-energy configurations with less bonding character. This gives lower, usually noninteger, bond orders. Further discussion of bond order in transition metal complexes can be found in (*2*).
19. All manipulations were carried out under anaerobic and anhydrous conditions. With rapid stirring, a solution of {Cr(μ-Cl)Ar′}$_2$ [1.94 g, 2 mmol, synthesized in 70% yield from a [1:1] tetrahydrofuran (**THF**, 30 mL) solution of $CrCl_2(THF)_2$ and LiAr′ (*15*)] was added dropwise to a suspension of freshly prepared KC_8 (0.68 g, 5.0 mmol) in THF (15 mL) being cooled with an ice bath. The resulting dark suspension in a red solution was stirred for 16 hours to ensure complete reduction. The volatile materials were removed under reduced pressure, and a toluene (25 mL) extract of the solid was filtered, reduced to ~15 mL, and left for 2 days at 7°C, after which 0.74 g (41% yield) could be isolated as dark red, x-ray–quality crystals with decomposition > 200°C. Ultraviolet (UV)/visible (vis) [hexanes, given as maximum wavelength (λ_{max}) in nm and, in parenthesis, ε in mol^{-1} L cm^{-1})] is 488 (3200). Infrared (nujol) frequency ν – 1261s, 1092s, 1020s, 867w, 799s, 493w cm^{-1}, where s is strong and w is weak. Magnetic susceptibility χ_{corr} – 0.00112 emu/mol Cr at 2 to 300 K combustion analysis (We found the following: **C**, 80.72%; **H**, 8.01%. Calculation for $C_{110}H_{124}Cr_2$ gave: **C**, 80.14%; **H**, 8.29%).
20. Crystal data for **1**• $2C_7H_8$ were obtained at 90(2) **K** with use of a Bruker SMART 1000 diffractometer and MoK_α radiation (λ – 0.71073 Å). The crystal data are as follows: *a* – 9.9982(15) Å, *b* – 10.8869(16) Å, *c* – 14.410(2) Å, α – 88.641(2)°, β – 82.242(3)°, γ – 76.129(2)°, triclinic, *Z* – 1, R_1 for 7931 [data intensity $I > 2\sigma(I)$] data – 0.0320, wR_2 (all data) – 0.0939.
21. For magnetic measurements, the samples were sealed under N_2 in 2-mm quartz tubing. The sample magnetization was measured with use of a Quantum Design MPMSXL7 superconducting quantum interference device (SQUID) magnetometer. For each measurement, the sample was zero-field cooled to 5 K, and the

magnetization was measured as a function of field to 2 T. The field was then reduced to 1 T, and the magnetization of the sample was measured in 5-K increments to 300 K. The observed susceptibility was corrected for the 0.000374–emu/mol Cr diamagnetic contribution to the susceptibility, a correction which was obtained from tables of Pascal constants. The extreme air sensitivity of 1 results in 2 to 6% contamination of all samples examined so far with paramagnetic impurities that involve Cr in higher oxidation states. It is also possible that the paramagnetic impurities could arise from incomplete or over reduction of $\{Cr(\mu\text{-}Cl)Ar'\}_2$. A plot of the molar magnetic susceptibility versus temperature for **1** is given in (*37*).

22. A different type of quintuple bond, which consists of three electron-pair bonds and four one-electron bonds, has been calculated to exist in U_2 molecules (*23*).
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25. The secondary Cr–C interaction involving a flanking aryl ring is due in part to the electron deficiency of the Cr center (12 electrons) and the electropositive character of the metal. It is also probable that the secondary Cr–C interaction lengthens the Cr–Cr bond because the "extra" Cr–C interaction competes for the chromium orbitals, thereby weakening the Cr–Cr bonding. Furthermore, all the Cr–C interactions occur in one plane so that the Ar′ ligands are eclipsed, which increases steric congestion and causes the Cr–Cr bond to lengthen. The existence of the eclipsed structure in 1 and its absence in the corresponding Ar′FeFeAr′ and Ar′CoCoAr′ species are consistent with the presence of δ bonding. The possibility that 1 featured bonds between chromium and hydrogen (i.e., an Ar′-substituted Cr(II) hydride derivative) was also entertained. No evidence for a Cr-H moiety could be observed in the infrared (IR) or ^{1}H nuclear magnetic resonance (NMR) spectrum of **1**. X-ray data and computational studies (*35*) also supplied no indication of the presence of the hydrogens near Cr.
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36. The electronic structure of **1** was calculated using DFT at restricted level (B3LYP/6-31*). Calculations were also performed using pure BP86 and BLYP functionals, which yielded very similar results. Additional DFT calculations carried out on 1 using the unrestricted Kohn-Sham broken symmetry (UKS-BS) approach yielded a wave function corresponding to a singlet diradical ground state with antiferromagnetic coupling between the two electrons that occupy the HOMO orbital. Other details of the DFT calculations are reported in (*35*).
37. Materials and methods are available as supporting material on *Science* Online.
38. We are grateful to the donors of the Petroleum Research Fund administered by the American Chemical Society and NSF for financial support of this work. In addition, we thank P. Klavins and L. D. Pham of the Department of Physics at the University of California, Davis, for recording the magnetic data, and C. R. Landis and E. Sinn for useful discussions. Metrical data for compound 1 are freely available from the Cambridge Crystallographic Database Centre (CCDC – 276888).

Supporting Online Material
www.sciencemag.org/cgi/content/full/1116789/DC1
Materials and Methods
Figs. S1 to S3
Tables S1 to S16
References and Notes

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A Direct Role for Dual Oxidase in *Drosophila* Gut Immunity

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Because the mucosal epithelia are in constant contact with large numbers of microorganisms, these surfaces must be armed with efficient microbial control systems. Here, we show that the *Drosophila* nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme, dual oxidase (dDuox), is indispensable for gut antimicrobial activities. Adult flies in which *dDuox* expression is silenced showed a marked increase in mortality rate even after a minor infection through ingestion of microbe-contaminated food. This could be restored by the specific reintroduction of dDuox, demonstrating that this oxidase generates a unique epithelial oxidative burst that limits microbial proliferation in the gut. Thus, oxidant-mediated antimicrobial responses are not restricted to the phagocytes, but rather are used more broadly, including in mucosal barrier epithelia.

The innate immune system provides an essential means of host defense in eukaryotes against a broad spectrum of microorganisms (*1*), and the production of microbicidal reactive oxygen species (ROS) is a key feature of this protective response (*2–6*). To date, most studies have focused on the molecular mechanism of respiratory burst in the professional phagocytes in response to microbial infection (*2, 6*). In contrast, the oxidant-dependent antimicrobial properties in mucosal epithelia, which are in permanent contact with the microbial realm, remain largely unknown. In *Drosophila*, the nuclear factor κB (NF-κB) pathways are critical during systemic infection (*7–11*) but appear to be less than crucial for host survival after epithelial infection (*12*). Natural gut infection has been associated with the rapid synthesis of ROS (*12*), and the dynamic cycle of ROS generation and elimination appears to be vital in *Drosophila*, because flies that lack ROS-removal capacity have an increased mortality (*12*). Such observations suggest an important role for ROS generation in controlling epithelial infection.

To directly examine if the epithelial oxidative burst system is required for host survival, we tested the potential superoxide-producing activity of intestinal epithelia in vitro (*13*). A basal level of superoxide generation was maintained in the membrane fraction of dissected intestines, and this increased markedly in the presence of calcium in a dose-dependent manner (Fig. 1A). Treatment with EGTA, or diphenylene iodonium (DPI), which is a flavoprotein inhibitor that also inhibits the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase–dependent oxidative burst, completely blocked calcium-activated intestinal superoxide-producing activity (Fig. 1A). In humans, phagocytic cells generate the ROS precursor, superoxide, via the phagocytic oxidase (phox) complex (*2, 5*). Recently, the human genome has been shown to contain several NADPH oxidase family members [currently designated the Nox 1-5 and dual oxidase (Duox) 1-2], each of which is homologous to the phox catalytic subunit, $gp91^{phox}$/Nox2 (*14, 15*). The Duox family can be distinguished from the Nox family based on the presence of an N-terminal extracellular peroxidase-homology domain (PHD) in addition to the $gp91^{phox}$-like oxidase domain (*14, 15*). The Nox/Duox family of enzymes are expressed in a variety of nonphagocytic cells, suggesting that they require oxidase functions similar to those of $gp91^{phox}$/Nox2 (*16–18*). Recently, Duox has been shown to be expressed in the barrier epithelia, including epithelial cells of mucosal surfaces of colon, rectum, salivary gland ducts, and bronchi (*18–20*), and it has been suggested that Duox may provide an epithelial ROS source in host defense (*18–20*). To determine the in vivo role of *Drosophila* Nox and Duox homologs (dNox and dDuox, respectively) (fig. S1) with regard to epithelial immunity, we generated a set of loss-of-function trans-

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genic flies carrying specific *RNAi* (RNA interference) constructs (fig. S2) (*13*). Natural infection experiments revealed that ubiquitous expression of *dDuox-RNAi* resulted in a consistently increased mortality rate for a number of microorganisms (Fig. 1B and fig. S3). A similar result was obtained when *dDuox-RNAi* was restricted to the intestine (by using *cad-GAL4* driver), but not when it was introduced in the fat body/hemocytes—the main immune tissues in systemic immunity—by using *c564-GAL4* driver (Fig. 1B) (*13*). In contrast, the transgenic flies carrying the *UAS-dNox-RNAi*, as well as NF-κB pathway mutant flies, were completely unaffected by gut infection (Fig. 1B and fig. S4). The dDuox-dependent ROS did not affect the NF-κB–dependent antimicrobial peptide gene expression in the gut (fig. S5), and conversely, the NF-κB pathway was not involved in the basal and infection-induced expression of intestinal *dDuox* (fig. S6). The infection-inducible nature of the intestinal *dDuox* also suggests that the transcriptional control of *dDuox* may play an important role in the regulation of the intestinal ROS generation. Taken together, these observations demonstrate that intestinal dDuox plays a major role in host resistance during natural infection. In the intestinal membrane fraction of the *dDuox-RNAi* flies, measurable superoxide-producing activity was 30% of that observed in the control flies (Fig. 1C), indicating that dDuox provides the main source of ROS within the intestines. In addition, the basal and infection-induced levels of in vivo ROS measured from the dissected intestines of the *dDuox-RNAi* flies were also significantly lower than those observed in the control intestines (Fig. 1D) (*13*). In a control experiment, *immune-regulated catalase (IRC)–RNAi* flies showed significantly increased basal and infection-induced ROS levels (Fig. 1D), which can be explained by a diminution of infection-induced ROS-removal capacity (*12*). Double knock-down flies carrying both *dDuox-RNAi* and *IRC-RNAi* exhibited normal levels of ROS (both basal and infection-induced ROS) (Fig. 1D) and displayed normal survival rates during natural infection (Fig. 1E). This phenomenon most likely reflects the fact that *dDuox-RNAi* used in the study resulted in a partial loss of function (fig. S2). The residual ROS level of *dDuox-RNAi* flies (Fig. 1, C and D) was apparently augmented to the normal control level by *IRC-RNAi* (Fig. 1D). Thus, the suppression of the infection-induced mortality of *dDuox-RNAi* flies by *IRC-RNAi* can be attributed to a reciprocal compensatory effect of the two opposing knock-down phenotypes. In theory, if we used adult flies carrying the *dDuox* null allele that show a total absence of a basal level of ROS, reducing the expression of *IRC* with *IRC-RNAi* may not protect the *dDuox* null mutant flies from infection. However, we could not reduce *dDuox* expression further using more effective *dDuox-RNAi* lines to confirm this possibility because of their larval/pupal lethality even in the presence of *IRC-RNAi*. At present, we cannot rule out the possible existence of other minor ROS-generating enzyme(s) in the adult gut. However, because dDuox is the main enzyme system involved in de novo synthesis of infection-induced epithelial ROS during gut infection, the redox balance between "dDuox-dependent ROS generation" and "IRC-dependent ROS removal" appears to represent a principal determinant of host survival during natural infection.

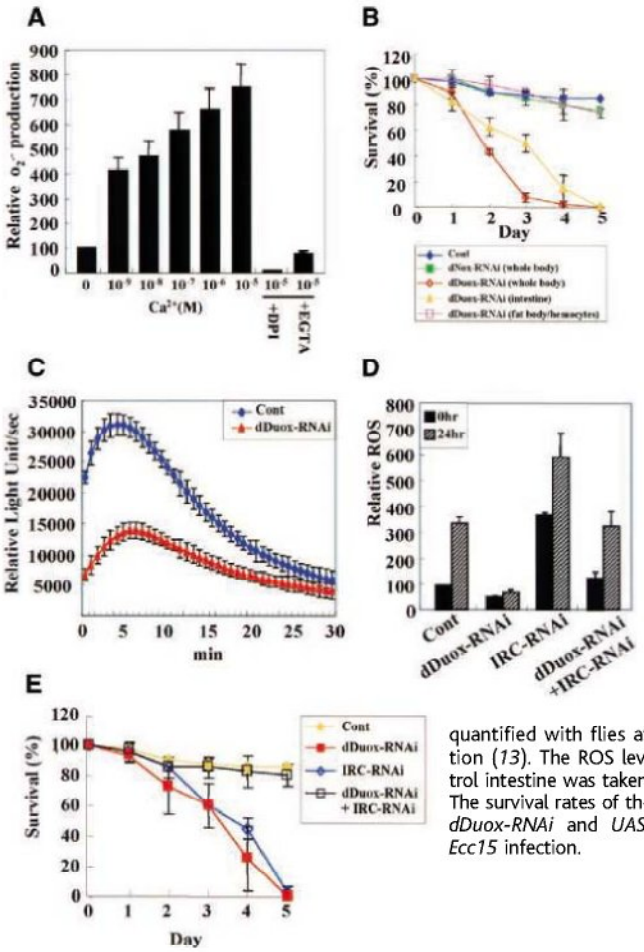
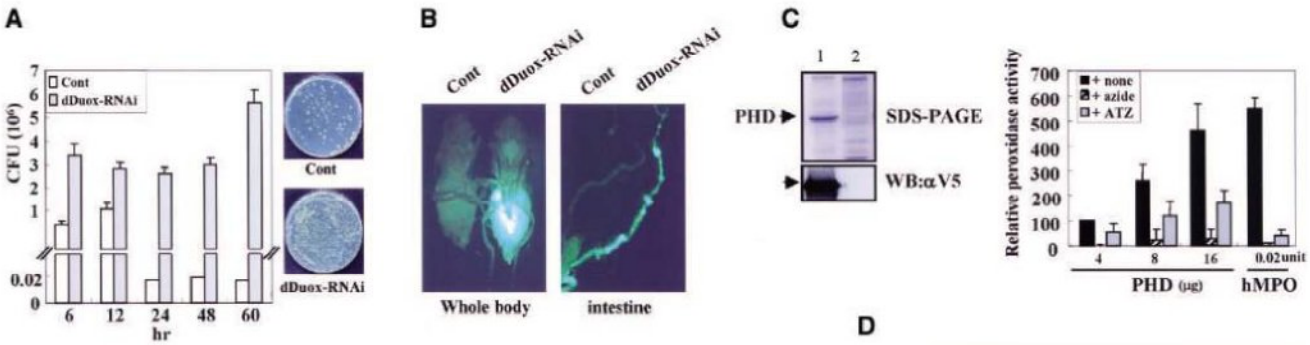


Fig. 1. Duox is responsible for infection-induced ROS generation, which is indispensable for host survival during natural gut infection. The genotypes of the flies used are indicated in the supporting text. All results are expressed as the mean ± SD of three different experiments. (**A**) Superoxide-generating activity in the intestinal membrane fraction. The superoxide production level in the intestine in the absence of calcium was taken arbitrarily to be 100. (**B**) Survival rates of *dDuox-RNAi* and *dNox-RNAi* flies were assessed after natural *Ecc15* infection. (**C**) Reduced in vitro intestinal superoxide-generating activity of *dDuox-RNAi* flies. (**D**) Reduced in vivo ROS of *dDuox-RNAi* flies. The total intestinal ROS levels were quantified with flies after natural *Ecc15* infection (*13*). The ROS level in the uninfected control intestine was taken arbitrarily to be 100. (**E**) The survival rates of the flies carrying both *UAS-dDuox-RNAi* and *UAS-IRC-RNAi* after natural *Ecc15* infection.

To investigate the in vivo relation between Duox activity and microbial persistence, we next examined the intestines of *dDuox-RNAi* flies during natural infection, using a green fluorescence protein (GFP)–tagged pathogen (*13*). Notably, unlike normal flies, ingested bacteria were shown to persist and proliferate throughout the intestinal tracts of the *dDuox-RNAi* flies [~300 times as many colony-forming units (CFUs) as those of the control intestines at 60 hours after infection] (Fig. 2, A and B). These results demonstrate that dDuox plays an important role in limiting the extent of microbial proliferation within the gut.

In mammals, the gp91^{phox}/Nox2-like oxidase domain of Duox has been reported to generate superoxide that is spontaneously dismutated to H_2O_2 after being secreted to the extracellular compartment (*14*, *18*). However, it has also been shown that H_2O_2 is only microbicidal at high concentrations and that exogenously generated superoxide does not directly kill microbes (*3*, *21*). Therefore, a variety of secondary oxidants have been proposed to account for the destructive capacity of phagocytes in mammals (*3*, *6*). One such secondary oxidant is the highly microbicidal HOCl, which is generated from H_2O_2 by neutrophil-derived myeloperoxidase (MPO) in the presence of chloride (*3*, *4*, *6*). Given that the PHD of dDuox exhibited significant in vitro peroxidase activity (Fig. 2C) (*13*), we tested whether H_2O_2 can also be used by the PHD to generate secondary oxidant of higher antimicrobial activity (*2*, *6*). The chloride-dependent microbicidal activity assay (*13*) showed that the recombinant PHD displays a significantly amplified antimicrobial effect, but only in the presence of both chloride and H_2O_2 (Fig. 2D).



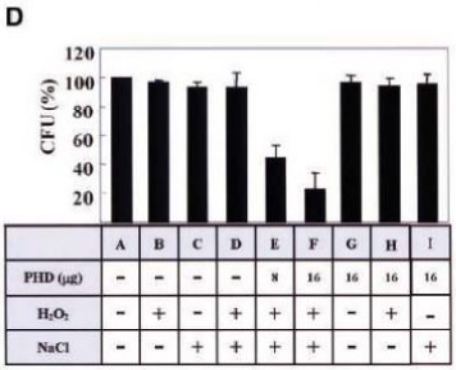
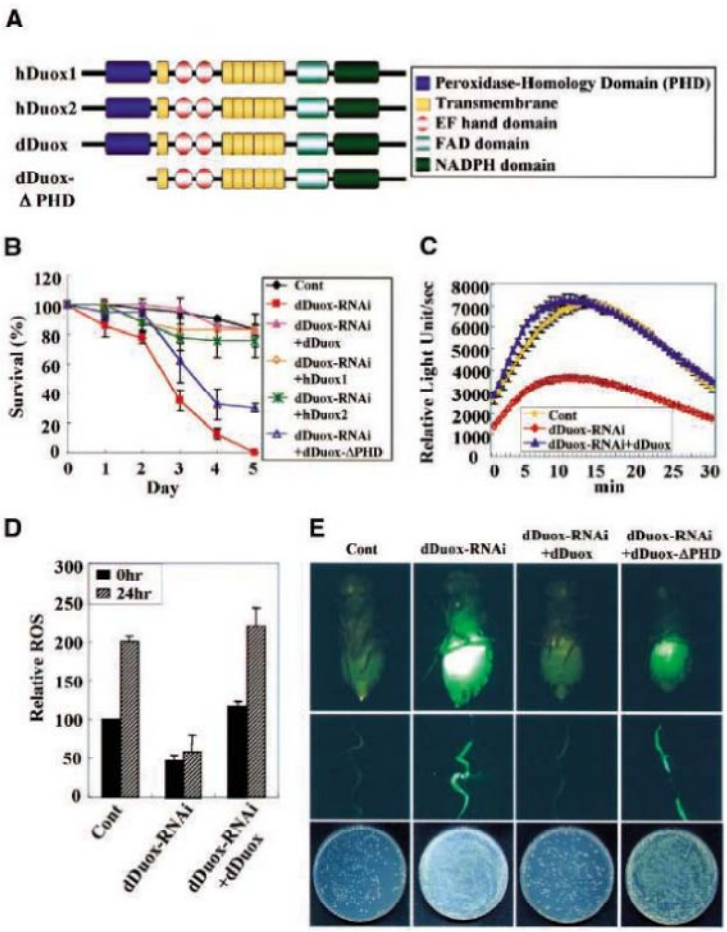


Fig. 2. Duox is responsible for limiting the onset of microbial proliferation in the gut. The genotypes of the flies used are indicated in the supporting text. All results are expressed as the mean ± SD of three different experiments. (**A**) Bacterial persistence in the guts of *dDuox-RNAi* flies. Bacterial persistence was measured using spectinomycin-resistant *Ecc15-GFP* (*13*). Time-course analysis (left) and representative plates of *Ecc15-GFP* recovered (60 hours after infection) from the intestines (right). (**B**) Incomplete *Ecc15-GFP* clearance in the *dDuox-RNAi* flies. Representative image of naturally infected flies and the dissected guts (60 hours after infection). (**C**) The peroxidase activity of the recombinant PHD of dDuox. SDS–polyacrylamide gel electrophoresis and Western blot analyses of the His/V5-tagged recombinant protein purified from the culture medium of S2 cells stably expressing the recombinant PHD (lane 1) or from the culture medium of control S2 cells (lane 2). The purified recombinant PHD proteins were then subjected to in vitro peroxidase activity (*13*). Human MPO was used as a positive control. The peroxidase inhibitors, aminotriazol (ATZ) and azide, were used (10 mM). The values were expressed as relative peroxidase activity, with the activity of the PHD (4 μg) arbitrarily set to 100. (**D**) Microbicidal activity of PHD. The chloride-dependent microbicidal activity was performed in the presence of H_2O_2 (*13*). The values were expressed as relative CFUs, with the number of CFU in the untreated bacteria arbitrarily set to 100%.

Fig. 3. The immune susceptibility of *dDuox-RNAi* flies can be markedly ameliorated by the reintroduction of either *dDuox* or *hDuox*. The genotypes of the flies used are indicated in the supporting text. All results are expressed as the mean ± SD of three different experiments. (**A**) Schematic presentation of various *Duox* constructs used for the generation of transgenic flies. (**B**) Rescue experiment. The *dDuox-RNAi* flies were crossed with flies carrying a variety of Duox constructs to determine the survival rates after natural *Ecc15* infection. (**C** and **D**) Reintroduction of *dDuox* into the *dDuox-RNAi* flies resulted in the complete restoration of in vitro intestinal superoxide-generating activity (C) and total in vivo intestinal ROS levels (D). The ROS levels in the uninfected control intestines were arbitrarily set at 100. (**E**) Persistence of *Ecc15-GFP* in the guts of *dDuox-RNAi* flies is completely abolished by reintroduction of *dDuox*, but not upon *dDuox-ΔPHD* expression. Representative images of naturally infected flies (top) and dissected intestines (middle), and representative plates of *Ecc15-GFP* recovered from the intestines (bottom), at 60 hours after infection.



These data demonstrate that the PHD exerts an MPO-like activity, which induces bacterial death in a chloride-dependent manner. Furthermore, we found that the levels of oxidative damage of ingested bacteria as assessed on the basis of protein carbonylation and lipid peroxidation (*13*) were significantly reduced in the intestines of the *dDuox-RNAi* flies as compared with those seen in the bacteria from control intestines (fig. S7). These results demonstrate that the infection-induced ROS generation by dDuox is responsible for the direct oxidative damage inflicted on ingested microbes.

To demonstrate that the immune susceptibility of *dDuox-RNAi* flies could indeed be attributed to the insufficient enzymatic activity of Duox, we attempted to protect *dDuox-RNAi* flies by reintroducing a variety of Duox enzymes [human Duox (hDuox) 1-2, dDuox, and dDuox-ΔPHD] (Fig. 3A). The reintroduction of both *hDuox* and *dDuox*, but not that of *dDuox-ΔPHD*, markedly augmented the survival rates of the *dDuox-RNAi* flies after natural infection (Fig. 3B). These results are consistent with the previous observations (Fig. 2D), indicating that PHD is required for the microbicidal effects of Duox. The reduced levels of in vitro superoxide-producing activities and of in vivo intestinal ROS in the *dDuox-RNAi* flies were almost completely restored to normal levels by reintroducing the *dDuox* (Fig. 3, C and D). Consistent with this, we detected that microbial persistence within the intestines of the *dDuox-RNAi* flies was reduced to control levels upon reintroduction of *dDuox*, but not upon *dDuox-ΔPHD* expression (Fig. 3E). Taken together, our results demonstrated that intestinal dDuox is responsible for the generation of infection-induced microbicidal ROS and that ROS thus generated are required for limiting the proliferation of local pathogens during gut-microbe interactions.

ROS perform a variety of functions in many biological events, including host defense, development, hormone biosynthesis, fertilization, and diverse intracellular signaling (*2–5*, *14*, *15*, *22–28*). In the present study, we have demonstrated the in vivo role of Duox in innate immunity via mediating epithelial oxidative burst in *Drosophila* gut. Our study broadens the concept of ROS-based immunity by demonstrating that the oxidant-dependent defense system is not restricted to the phagocytes but rather is found in barrier epithelia. In addition to the NF-κB pathway mediated defense system (*7–11*), the Duox-mediated ROS-dependent defense system involving both $gp91^{phox}$-like activity and MPO-like activity constitutes another microbicidal arm of *Drosophila* innate immunity. Further delineation of dDuox/IRC-mediated gastrointestinal redox homeostasis will provide important insight into innate immunity and the host-pathogen interaction.

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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S7
References

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Treatment of Autoimmune Neuroinflammation with a Synthetic Tryptophan Metabolite

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Local catabolism of the amino acid tryptophan (Trp) by indoleamine 2,3-dioxygenase (IDO) is considered an important mechanism of regulating T cell immunity. We show that IDO transcription was increased when myelin-specific T cells were stimulated with tolerogenic altered self-peptides. Catabolites of Trp suppressed proliferation of myelin-specific T cells and inhibited production of proinflammatory T helper–1 (T_H1) cytokines. *N*-(3,4,-Dimethoxycinnamoyl) anthranilic acid (3,4-DAA), an orally active synthetic derivative of the Trp metabolite anthranilic acid, reversed paralysis in mice with experimental autoimmune encephalomyelitis, a model of multiple sclerosis (MS). Trp catabolites and their derivatives offer a new strategy for treating T_H1-mediated autoimmune diseases such as MS.

Degradation of the essential amino acid Trp is now established to play an important role in immunity. The rate-limiting enzyme in Trp catabolism is IDO, and although IDO mRNA is ubiquitously expressed at low levels, IDO protein expression and enzymatic activity are tightly controlled (*1*). During inflammation, IDO is rapidly up-regulated in certain cell types by proinflammatory stimuli and T_H1 cytokines such as interferon-γ (IFN-γ) (*2*). Munn and Mellor first demonstrated a role for Trp catabolism in the control of immune responses in 1998 by showing that inhibition of IDO induces fetal allograft rejection in mice (*3*). Potentially, activated antigen-presenting cells (APCs) catabolize Trp to produce a local immunosuppressive environment able to control T cell homeostasis and self-tolerance during inflammation (*4*, *5*).

In addition to Trp depletion, there is increasing evidence that catabolites of Trp assume an active role in dampening allogenic proliferation and inducing apoptosis of activated T cells (*6*, *7*). These catabolites, collectively referred to as kynurenines (Kyns), are important in the dialogue between the immune system and the central nervous system (CNS) (*8*) and have emerged as an attractive target for drug development (*9*, *10*).

In multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE), autoreactive $CD4^+$ T_H1 cells secreting IFN-γ and tumor necrosis factor (TNF) drive inflammation, which is amplified

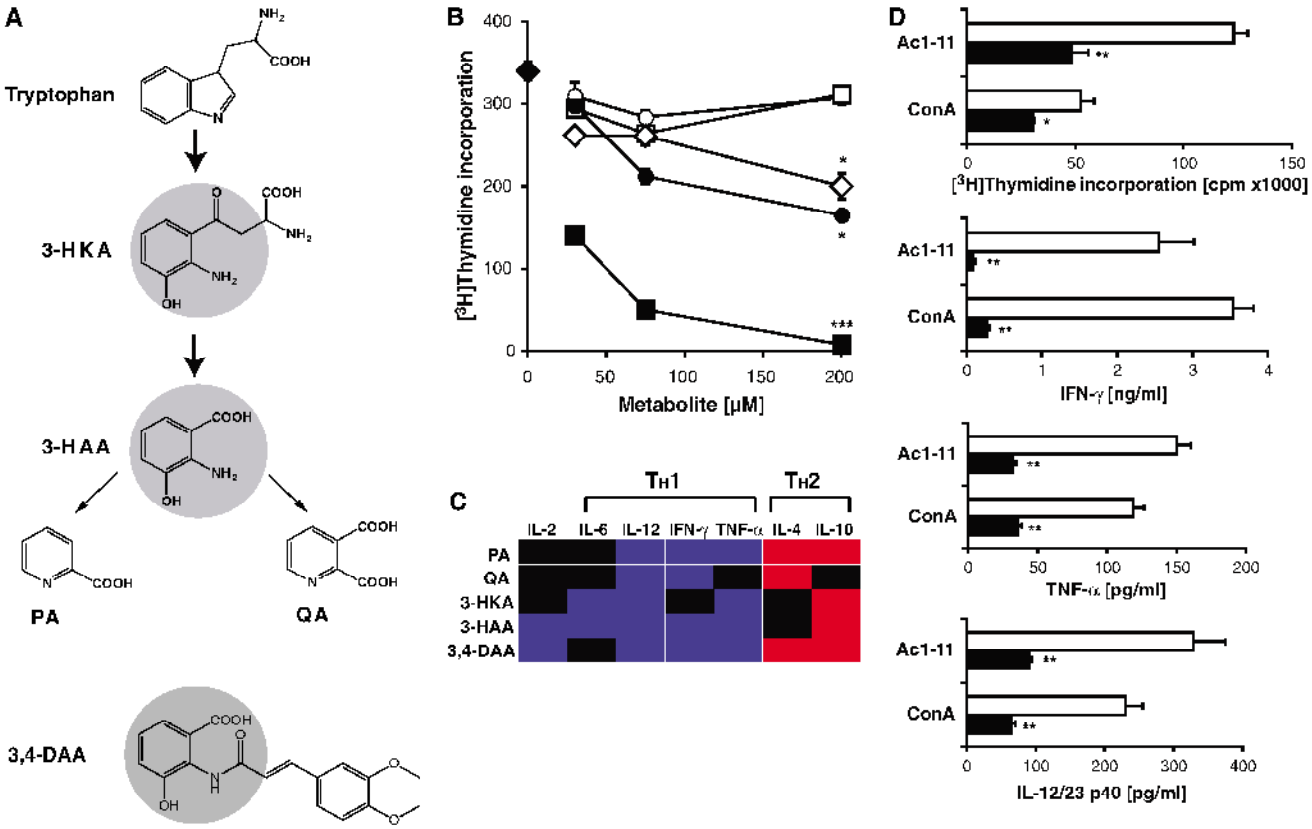

Fig. 1. Modulation of T cell proliferation by Trp metabolites. (**A**) Chemical structure of Trp metabolites. 3-HKA, 3-hydroxykynurenic acid; 3-HAA, 3-hydroxyanthranilic acid; PA, picolinic acid; QA, quinolinic acid; and 3,4-DAA. (**B**) Splenocytes from MBP Ac1-11 TCR transgenic mice were left untreated (control, ◆) or incubated with Trp metabolites PA (○), QA (□), 3-HKA (◇), 3-HAA (●), 3,4-DAA (■) at the concentrations indicated and stimulated with MBP Ac1-11 (5 μg/ml). Splenocytes were pulsed with [^{3}H]thymidine after 48 hours for 18 hours. Data represent mean counts per minute (cpm) and SEM of triplicates and are representative of four independent experiments. $*P < 0.05$, $***P < 0.001$. (**C**) Splenocytes from MBPAc1-11 TCR transgenic mice were activated with MBP Ac1-11 (0.5 to 2.5 μg/ml) in the absence or presence of Trp metabolites PA, QA, 3-HKA, 3-HAA, or 3,4-DAA at 200 μM (IL-2, IFN-γ, TNF-α, IL-6, and IL-12/23 p40) or 30 μM (IL-4, IL-10). Cytokine release was measured after 48 hours (IL-2, IL-6, IL-12/23 p40), 72 hours (IFN-γ, TNF-α), or 120 hours (IL-4, IL-10) by using enzyme-linked immunosorbent assay (ELISA, OptEIA Cytokine Sets, BD Pharmingen). Data are displayed as a heat map. Raw data are presented in table S2. (**D**) MBP Ac1-11 TCR transgenic mice ($n = 3$ per group) were fed with 3,4-DAA for 5 days (500 mg/kg per day). Pooled splenocytes of vehicle (Na-CMC)–treated (white bars) or 3,4-DAA–treated (filled bars) mice were stimulated with MBP Ac1-11 (5 μg/ml) or concanavalin A (ConA) (2 μg/ml) in vitro. Proliferation and cytokine analysis were performed as described. Mean values and SEM of triplicates are given, and data are representative of two independent experiments. $*P < 0.05$, $**P < 0.01$.

within the CNS by microglia (*11*, *13*). Experimental therapeutic approaches aimed at skewing the cytokine profile of myelin-specific T_H cells from T_H1 to T_H2 have included altered peptide ligands (APLs) (*14*, *15*), hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors ("statins"), and DNA vaccination combined with gene delivery of interleukin 4 (IL-4) (*16*–*18*). APLs are peptides modified at crucial receptor binding residues to modulate proliferation and the cytokine profile of antigen-specific T cells by altering the strength of T cell receptor (TCR) signaling (*14*). Deviation of cytokine responses toward T_H2 is also a mechanism of action of glatiramer acetate, an example of an APL, which is an approved treatment for multiple sclerosis (*19*, *20*). Consequently, APLs inducing a T_H2 immune response have emerged as a potential therapeutic strategy to induce self-tolerance to autoantigens and to treat autoimmune diseases such as MS (*21*–*24*). Such approaches aimed at inducing a T_H2 shift have been relatively promising in reversing ongoing paralytic disease in the EAE model.

Initially, we aimed at identifying gene transcripts that are differentially regulated in T cells treated with APL. To do this, a T cell line generated from mice with a transgenic TCR specific for the myelin basic protein (MBP) peptide Ac1-11 was used for a gene microarray analysis (*25*). The APL MBP Ac1-11[4Y] binds to the major histocompatibility complex (MHC) class II I-A^u with greater affinity than the native peptide. MBP Ac1-11 induces primarily a T_H1 response, whereas MBP Ac1-11[4Y] promotes a T_H2 response (*26*). Transcripts encoding IDO were more than 70-fold up-regulated after 48 hours in MBP Ac1-11[4Y] activated T cells compared with MBP Ac1-11–activated cells (*27*) (table S1). These data suggested a direct role of IDO in the tolerogenic effects of APLs.

We next tested the hypothesis that Trp metabolites (Kyns), generated by the enzymatic activity of IDO, play a role in regulating activation of myelin-specific T cells (*25*). Splenocytes isolated from MBP Ac1-11 TCR transgenic B10.PL mice were stimulated with

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MBP Ac1-11 in combination with the Trp metabolites picolinic acid (PA), quinolinic acid (QA), 3-hydroxyanthranilic acid (3-HAA), 3-hydroxykynurenic acid (3-HKA), and the synthetic derivative 3,4-DAA (*25*). 3,4-DAA was selected because it shares the anthranilic acid core with 3-HKA and 3-HAA (Fig. 1A) and because it is an orally active compound with favorable pharmacokinetics in humans (*28*). 3-HAA, 3-HKA, and 3,4-DAA suppressed antigen-specific proliferation of MBP Ac1-11 TCR transgenic $CD4^+$ T cells (Fig. 1B). Suppression of T cell response by 3,4-DAA was associated with a G_1/S-phase arrest in $CD4^+$ T cells rather than cytotoxic effects (fig. S1). Both natural Trp metabolites and 3,4-DAA reduced the release of IL-2, IFN-γ, and TNF-α from MBP Ac1-11 TCR transgenic T cells after antigen stimulation. Conversely, IL-4 and IL-10 were increased (Fig. 1C; table S2). Thus, both natural Trp metabolites and 3,4-DAA skew the cytokine profile of these T cells from T_H1 to T_H2.

To examine the effects of 3,4-DAA on myelin-specific T cells in vivo, MBP Ac1-11 TCR transgenic mice were fed with 3,4-DAA for 5 days (*25*) (supporting online text, note S1). Splenocytes isolated from these animals showed reduced MBP Ac1-11 specific proliferation. Similarly, antigen-induced release of IFN-γ, TNF-α, and IL-12/23 p40 was profoundly suppressed (Fig. 1D), which indicates that 3,4-DAA is active when given orally to suppress the activation of autoreactive T_H1 cells.

Adaptive immunity in $CD4^+$ T_H cells requires antigen presentation on MHC class II molecules and delivery of costimulatory signals via molecules such as CD40, CD80, and CD86, all inducible by IFN-γ (*29*). To assess the influence of Trp metabolites on antigen presentation, we used EOC20 microglial cells as a model (*25*). EOC20 cells express MHC class II and costimulatory molecules constitutively at low levels, and these cells rapidly upregulate on exposure to IFN-γ. 3,4-DAA decreased IFN-γ–induced cell surface expression of MHC class II and costimulatory molecules (Fig. 2A) (*30*). 3,4-DAA–mediated suppression of IFN-γ induced MHC class II expression in EOC20 cells was paralleled by an inhibition of the MHC class II transactivator (CIITA) (Fig. 2B). In addition, 3,4-DAA suppressed expression of inducible nitric oxide synthase (iNOS) and nitric oxide (NO) release from EOC20 cells induced by IFN-γ and lipopolysaccharide (LPS) (Fig. 2, C and D). On the basis of these results, we hypothesized that 3,4-DAA might interfere with IFN-γ signaling: Type I and type II interferons predominantly signal through phosphorylation of signal transducer and activator of transcription (STAT) 1α (*31*); and consistent with the previous results, phosphorylation of STAT1α induced by IFN-γ was suppressed after incubation with 3,4-DAA (Fig. 2E). 3,4-DAA also suppressed the activation of APCs in vivo (fig. S3, table S3).

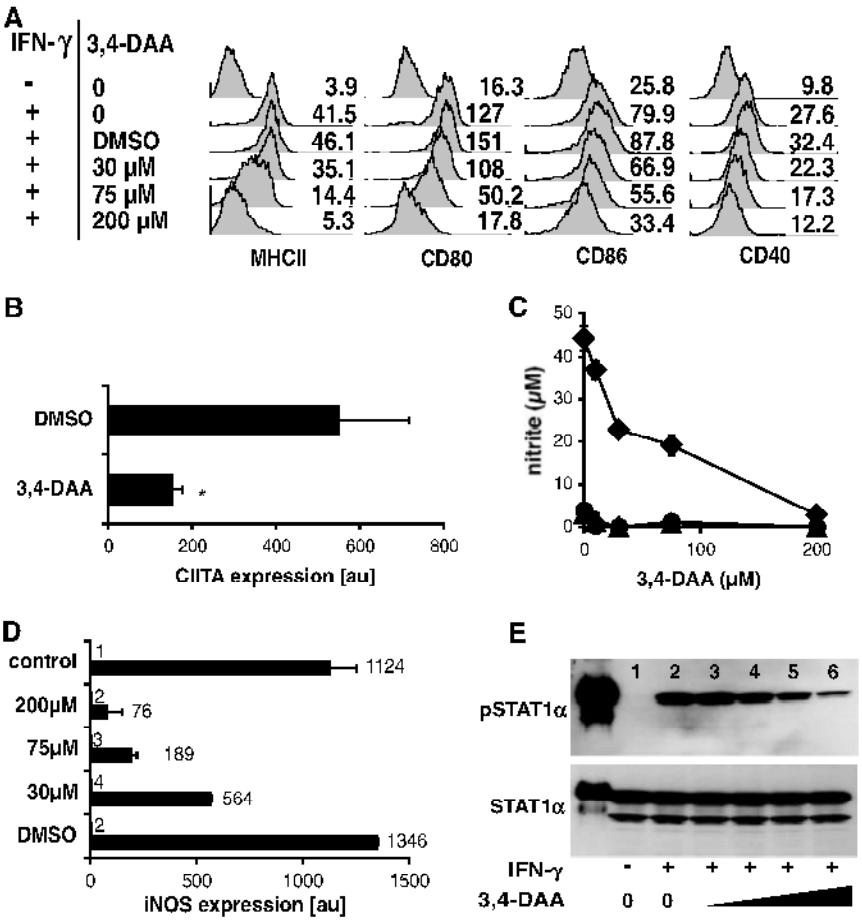

Fig. 2. Mechanisms of APC function modulated by 3,4-DAA. EOC20 cells were incubated with medium alone, dimethyl sulfoxide vehicle (DMSO), or 3,4-DAA at the concentrations indicated and were stimulated with IFN-γ (200 U/ml) and/or LPS (200 ng/ml). (**A**) Cell surface expression of MHC class II ($I\text{-}A^k$), CD40, CD80, and CD86 was determined after 48 hours using flow cytometry. Histograms are representative of three independent experiments. Values represent mean fluorescent indices. (**B**) RNA was extracted after 24 hours and reverse-transcribed. CIITA cDNA expression was quantified by using real-time polymerase chain reaction (PCR). Values represent mean arbitrary expression levels of triplicates and SEM normalized to expression of β-actin. Data are representative of two independent experiments. *$P < 0.05$. (**C**) Nitrite release of unstimulated (▲), IFN-γ–stimulated (●), or IFN-γ– and LPS-stimulated cells (◆) was determined after 48 hours by using the Griess assay. Values are mean nitrite concentration and SEM of triplicates and are representative of three independent experiments. (**D**) RNA was extracted after 24 hours and reverse-transcribed. iNOS cDNA expression was semiquantified by using PCR in real time. Values of unstimulated (white bars) and IFN-γ–stimulated (black bars) represent mean arbitrary expression levels of triplicates and SEM normalized to expression of β-actin. Data are representative of two independent experiments. (**E**) Western blot analysis of whole-cell protein extracted 15 min after stimulation with IFN-γ by using a phospho-specific STAT1α antibody. The membrane was reprobed with a non–phospho-specific STAT1α antibody to ensure equal loading.

To assess whether 3,4-DAA suppresses the function of autoreactive T_H1 cells, we tested the compound in vivo using the relapsing-remitting version of EAE, which serves as a model of relapsing-remitting MS (*25*). As patients with relapsing-remitting MS are typically treated after the first onset of clinical symptoms to prevent further attacks, we initiated treatment after the onset of disease. Although vehicle-treated animals displayed severe relapses throughout the course of disease, animals treated with 3,4-DAA had fewer and milder relapses and less severe disease (Fig. 3A). At multiple dose levels, there was significant reduction in clinical disease index (CDI) and peak relapse score (table S4).

Concordant with the capacity of 3,4-DAA to suppress activation of myelin-specific T_H1 cells in vitro, the frequency of activated T cells was decreased in mice treated with 3,4-DAA (Fig. 3B). Moreover, PLP-specific T cell proliferation and the release of IFN-γ, TNF-α,

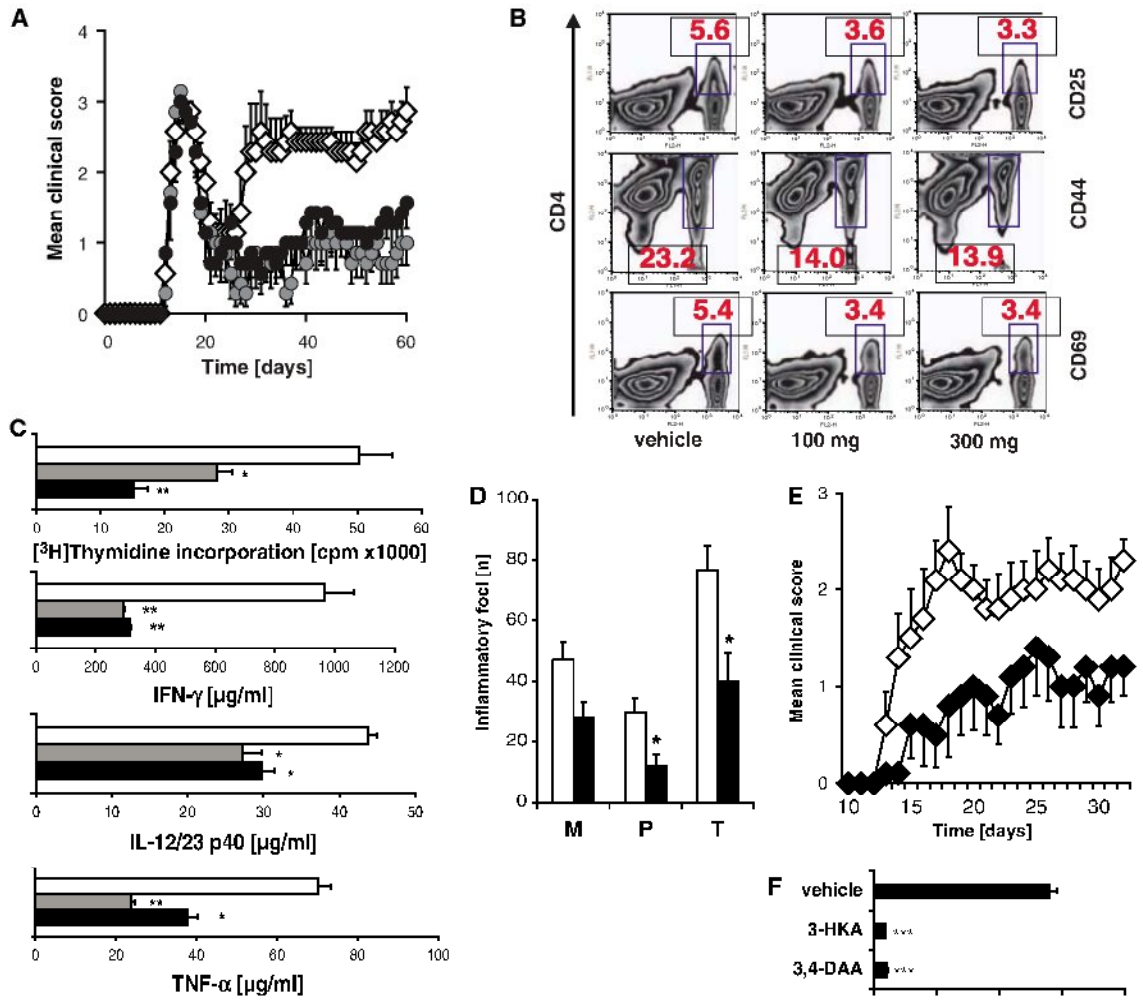

Fig. 3. 3,4-DAA ameliorates established EAE. Female SJL/J mice 7 to 8 weeks old were immunized with PLP p139-151. Treatment was initiated at the peak of clinical symptoms (day 16 to day 18) by oral gavage twice daily (3,4-DAA) or by intraperitoneal injections once daily (3-HKA). (**A**) Clinical scores of vehicle-treated mice (◇) and mice treated with 3,4-DAA at 100 mg/kg per day (●) or 300 mg/kg per day (●) (0, asymptomatic; 1, limp tail; 2, partial hind limb paresis; 3, hind limb paralysis; 4, quadriplegia; 5, moribund or dead) were assessed each day. Data represent mean scores (n = 9, vehicle and 300 mg/kg per day; n = 10, 100 mg/kg per day) and SEM. (**B**) Flow cytometric analysis of pooled splenocytes of vehicle-treated (n = 6) or 3,4-DAA–treated (n = 7, 100 mg/kg per day; n = 6, 300 mg/kg per day) mice isolated 60 days post immunizations. Values represent double-positive cells, that is, CD4$^+$ and CD69$^+$, CD44$^+$, or CD25$^+$. (**C**) Pooled splenocytes of vehicle-treated (n = 6, white bars), or 3,4-DAA–treated (n = 7, 100 mg/kg per day, gray bars; n = 6, 300 mg/kg per day, black bars) were isolated after 60 days and stimulated in vitro with PLP p139-151 (20 μg/ml). Proliferation was assessed as in Fig. 1B, except cells were pulsed after 72 hours of culture. Cytokines were analyzed as in Fig. 1C. Data represent mean values of triplicates and SEM. *P < 0.05, **P < 0.01. (**D**) Brains and spinal cords were extracted 60 days after immunization. Infiltration of inflammatory cells was counted in randomly chosen brains from vehicle-treated (n = 3, white bars) and 3,4-DAA–treated (n = 3, 100 mg/kg per day, black bars) by a neuropathologist blinded to the treatment. M, meningeal foci; P, parenchymal foci; T, total number of foci. Data represent mean number of inflammatory foci and SEM. *P < 0.05. (**E**) Lymph node cells from mice with EAE that had been treated with vehicle (◇) or 3,4-DAA (200 mg/kg per day, ◆) were stimulated in vitro with PLP p139-151 (10 μg/ml) and adoptively transferred into SJL/J recipient mice that had been immunized 8 days before adoptive transfer with PLP p139-151. Data represent mean clinical scores. (**F**) Lymph node cells from mice with EAE that had been treated with vehicle, 3,4-DAA (200 μg/kg per day), or 3-HKA (150 mg/kg per day) were stimulated in vitro with PLP p139−151 (10 μg/ml). Cytokines were analyzed as in Fig. 3C. Values are displayed as amounts of cytokines released by cells stimulated with PLP p139-151 divided by amounts of cytokines released by unstimulated cells. Data represent mean values and SEM. **P < 0.01, ***P < 0.001.

and IL-12/23 p40 were decreased after treatment with 3,4-DAA (Fig. 3C) (SOM text, note S2). Next, assessment of brains and spinal cords of mice with EAE revealed a reduction of parenchymal and total inflammatory foci in CNS tissue from mice treated with 3,4-DAA compared to vehicle-treated mice (Fig. 3D).

We evaluated whether activation of APCs in the CNS was suppressed in vivo (25, 32). Consistent with previous results, expression of MHC class II, CD40, CD80, CD86, and iNOS was drastically reduced in spinal cord microglial cells when mice with EAE had been treated with 3,4-DAA (Fig. 4A). Moreover, the expression of the CIITA, iNOS, TNF-α, and IL-12/23 p40 genes was reduced in spinal cords of animals treated with 3,4-DAA (Fig. 4B). Taken together, these results indicate suppression of APCs as a key mechanism underlying the immunosuppressive effects of 3,4-DAA.

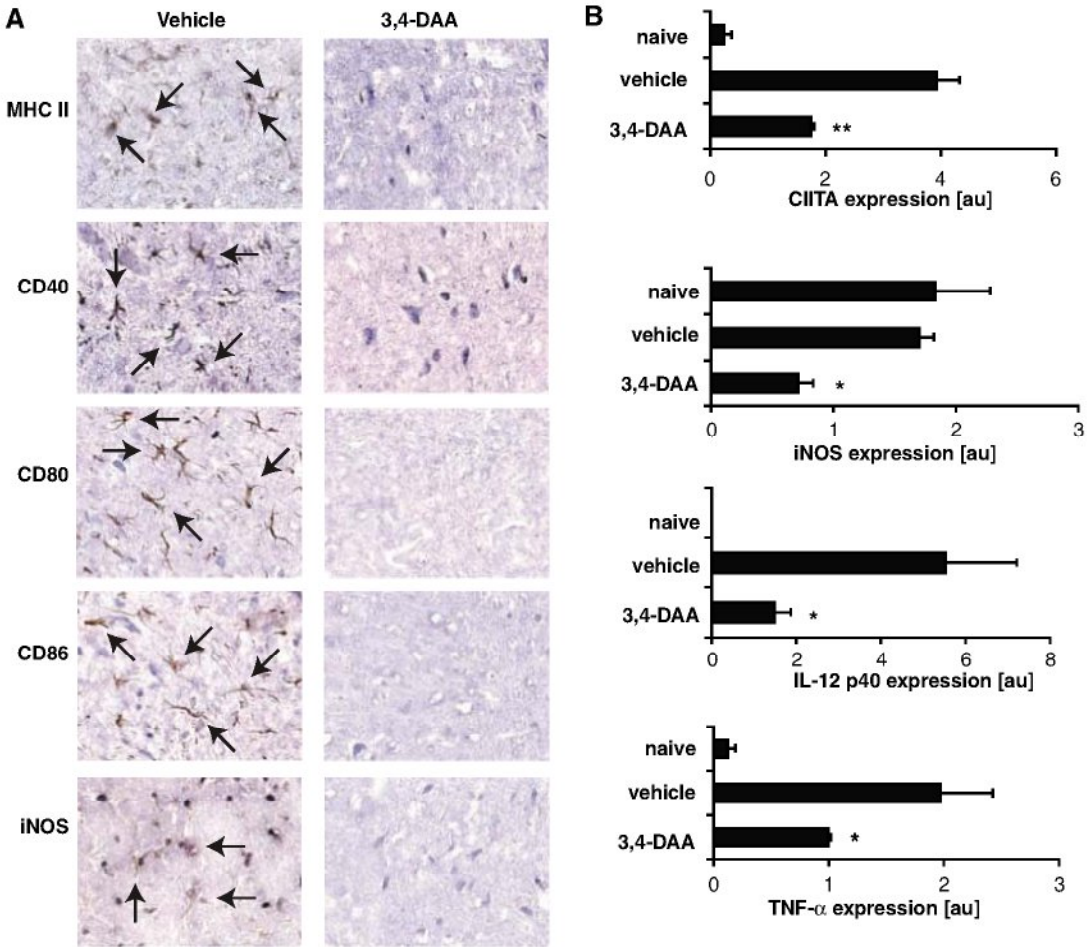

Fig. 4. 3,4-DAA suppresses the activation of CNS antigen-presenting cells in EAE. (**A**) Female SJL/J mice 7 to 8 weeks old were immunized with PLP p139-151 two days after the initiation of treatment with 3,4-DAA (200 mg/kg per day) by oral gavage twice daily. 12 days after immunization, brains and spinal cords were removed and stained for MHC class II (I-A^k), CD40, CD80, CD86, and iNOS. (**B**) Female SJL/J mice 7 to 8 weeks old were immunized with PLP p139-151. Treatment with 3,4-DAA was initiated at day 16. Naïve animals served as a control. RNA was isolated from spinal cords 60 days after immunization ($n = 3$). After reverse transcription, cDNA expression of the indicated transcripts was analyzed by using real-time PCR. Values represent mean arbitrary expression levels of triplicates and SEM normalized to expression of β-actin. Data are representative of three independent experiments. $^*P < 0.05$, $^{**}P < 0.01$.

We finally assessed whether suppression of myelin specific T_H1 cells results from direct inhibition of these cells by 3,4-DAA, or whether this suppression might be mediated by myelin-specific T_H2-like cells induced by treatment with the drug (*25*). Myelin-reactive lymph node cells from donor mice treated with 3,4-DAA after onset of EAE delayed the onset and ameliorated the symptoms of EAE when adoptively transferred into recipient mice that had been immunized with PLP p139-151 (Fig. 3E). Finally, administration of either the natural Trp metabolite 3-HKA or the synthetic Trp metabolite 3,4-DAA to SJL mice with EAE induced release of IL-10 from PLP p139-151 reactive lymph node cells (Fig. 3F) (*25*). Induction of IL-10 with these compounds in myelin-specific T cells was also observed in vitro (Fig. 1C). Collectively, these data indicate that natural Trp metabolites induce antigen-specific IL-10–producing T cells with regulatory potential in vitro and in vivo. Moreover, these findings strongly suggest that 3,4-DAA, structurally related to Trp metabolites, has similar physiologic effects as well.

Interestingly, the structurally related immune regulators, linomide and laquinimod with a yet unidentified mechanism of action, are quinoline carboxamides with structural homology to these same Trp metabolites (fig. S5). Both linomide and laquinimod are effective in treating EAE and show some efficacy in patients with MS (*33*, *34*). Quinoline carboxamides, however, are cardiotoxic. Thus, trials of linomide in patients with MS have been halted. These observations further support our findings that Trp metabolites may represent a novel class of drugs effective in treating T_H1-mediated autoimmune diseases. Our results indicate that 3,4-DAA, by acting as a mimetic of the Trp catabolites, can replace the physiologic role of natural Trp catabolites in inhibiting autoreactive T_H1 cells. Orally active derivatives of Trp metabolites, such as 3,4-DAA, may thus represent a new class of drugs for therapy to treat ongoing T_H1-mediated autoimmune diseases.

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Regulated Pole-to-Pole Oscillations of a Bacterial Gliding Motility Protein

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Little is known about directed motility of bacteria that move by type IV pilus–mediated (twitching) motility. Here, we found that during periodic cell reversals of *Myxococcus xanthus*, type IV pili were disassembled at one pole and reassembled at the other pole. Accompanying these reversals, FrzS, a protein required for directed motility, moved in an oscillatory pattern between the cell poles. The frequency of the oscillations was controlled by the Frz chemosensory system, which is essential for directed motility. Pole-to-pole migration of FrzS appeared to involve movement along a filament running the length of the cell. FrzS dynamics may thus regulate cell polarity during directed motility.

Gliding motility is important for bacterial movement on solid surfaces, virulence, and development (*1*). Twitching motility in *Pseudomonas aeruginosa* or social motility (S-motility) in *Myxococcus xanthus* involves assembly of type IV pili at the leading end of cells. Motion is produced as the fibers bind to receptors on the substratum, or another cell, and retract (*2*). Control of directional movements requires periodic cell reversals, which are regulated by chemosensory systems (*1*, *3*, *4*). It has been proposed that cellular reversals are achieved by switching the sites of pili extrusion from one cell pole to the other (*4*).

In *M. xanthus*, directed motility allows cells to coordinate movements toward nutrients or, when limiting, fruiting bodies (*5*). FrzS is required for S-motility–dependent vegetative swarming. It contains an N terminal receiver like domain, an alanine-proline–rich linker, and an extended coiled-coil domain (fig. S1A) (*6*). *frzS* mutants are impaired in S-motility swarming because they are defective in regulating pili–mediated directional movements (fig. S1, B and C). Indeed, mutants that have strong directional defects cannot swarm or form fruiting bodies (*7*, *8*).

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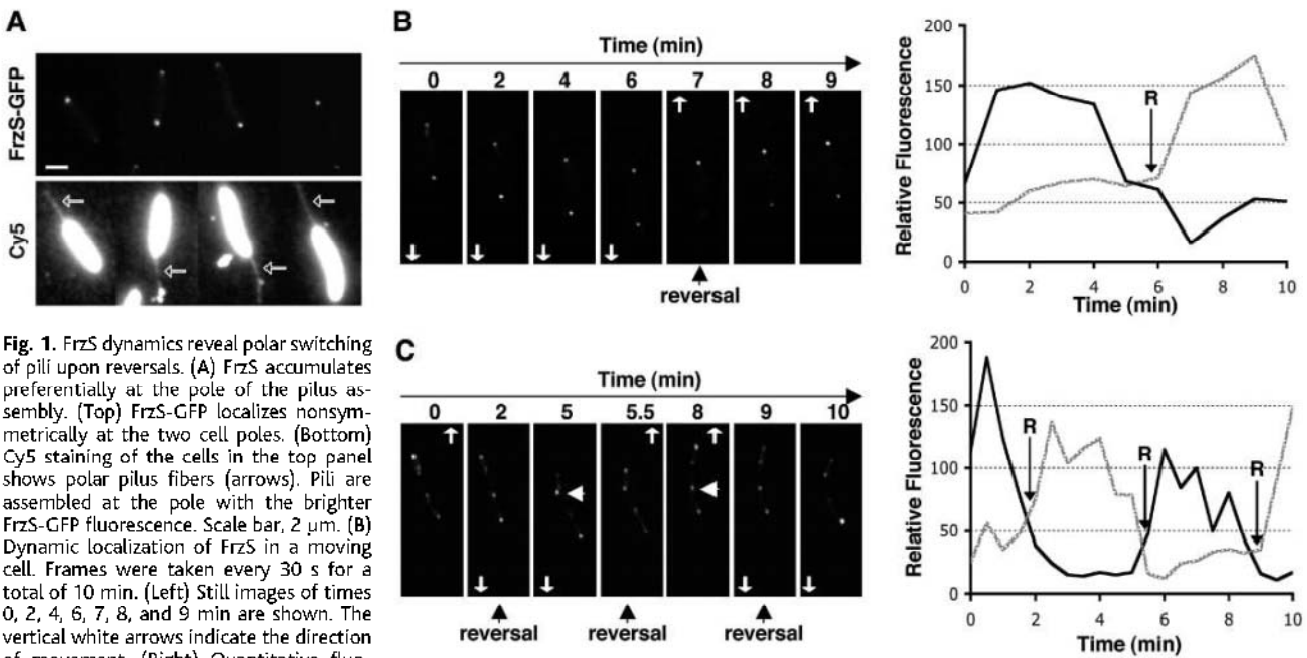

Fig. 1. FrzS dynamics reveal polar switching of pili upon reversals. (**A**) FrzS accumulates preferentially at the pole of the pilus assembly. (Top) FrzS-GFP localizes nonsymmetrically at the two cell poles. (Bottom) Cy5 staining of the cells in the top panel shows polar pilus fibers (arrows). Pili are assembled at the pole with the brighter FrzS-GFP fluorescence. Scale bar, 2 μm. (**B**) Dynamic localization of FrzS in a moving cell. Frames were taken every 30 s for a total of 10 min. (Left) Still images of times 0, 2, 4, 6, 7, 8, and 9 min are shown. The vertical white arrows indicate the direction of movement. (Right) Quantitative fluorescence analysis of the cell presented at left. The relative fluorescence intensities (arbitrary units) of each cell pole were measured and plotted over time. Black line, initial leading pole; gray line, initial trailing pole; R, Reversal. (**C**) Dynamic localization of FrzS in a *frzCD*^{c} mutant cell. Frames were taken every 30 s for a total duration of 10 min. (Left) Still images of times 0, 2, 5, 5.5, 8, 9, and 10 min are shown. The vertical white arrows indicate the direction of movement. Horizontal white arrows point to *frzCD*^{c} –dependent additional FrzS spots that brighten immediately before switch of the bright pole. (Right) Quantitative fluorescence analysis of the cell presented at left. Labels as in (**B**).

To investigate the cellular localization of FrzS, we constructed a strain containing green fluorescent protein (GFP) in a chimeric *frzS-gfp* gene in place of the endogenous *frzS* gene (fig. S2A). The FrzS-GFP fusion protein was stably expressed and functional (fig. S2, B and C). FrzS-GFP was localized in patches primarily at both cell poles (Fig. 1A). In many cells, one pole was bright and the other dim, which suggested that FrzS accumulated at one pole in preference to the other (Fig. 1A). The brighter FrzS-GFP pole usually corresponded to the pole containing pili (37 of 40 analyzed cells), visualized with Cy5 (Fig. 1A), a nonspecific fluorescent dye that has been used to label pilus fibers in *P. aeruginosa* (*9*). We followed individual cells expressing FrzS-GFP as they moved on agar pads by time-lapse fluorescence microscopy. As expected, the brighter FrzS-GFP fluorescent patch was typically observed at the leading end of the cell (Fig. 1B and Table 1).

Table 1. Correlation between FrzS localization pattern and cellular reversals. Total cells is the number of filmed cells for each strain. Bright leading poles were unambiguously asymmetrical with a brighter patch at the leading pole. Dim leading poles were unambiguously asymmetrical with a dimmer patch at the leading pole. No symmetry means cells displayed movement with no evident FrzS asymmetry. A reversal was scored each time a cell changed its direction by 180°. A switch was scored each time a brighter pole became a dimmer pole. Percent correlation reflects the percentage of cells where a switch occurred concurrently with a reversal.

Measure	Background		
	WT	*frzE*	*frzCD*^{c}
Total cells	92	94	34
Scored cells			
Bright leading pole	86	84	34
Dim leading pole	4	5	0
No asymmetry	2	5	0
Reversals	33	8	92
Switch	33	0	92
Percent correlation	100	0	100

If cellular reversals involve pole-to-pole switching of pili fibers, then we should see relocalization of the brighter FrzS-GFP fluorescent patch from one cell pole to the other when a cell reverses. As predicted, fluorescence at the leading pole slowly decreased and gradually increased at the trailing pole so that the intensity was equalized at both poles after 5 to 6 min (Fig. 1B; movie S1). Fluorescence at the leading pole then dispersed, and the cell reversed direction causing the old trailing pole to become both the brighter pole and the new leading pole. FrzS-GFP fluorescence increased rapidly at the new leading pole as the cell continued to move in the new direction (Fig. 1B). Thereafter, fluorescence at the leading pole decreased as the trailing pole showed increased fluorescence. We tracked 92 cells for 10-min intervals and observed 33 cells reversing; cell reversal was always accompanied by relocalization of the brighter fluorescent patch from the old leading cell pole to the new leading cell pole (Table 1). Thus, pili switched poles when cells reversed, and FrzS was associated with the regulation of the switch.

The Frz chemosensory pathway acts as a biochemical regulator of cell reversals (*10*). *frz* mutants very rarely reverse their direction of movement and are defective in swarming and fruiting body formation (*11*). We monitored FrzS-GFP oscillations in a *frz*-null mutant (*frzE*) to determine whether the Frz pathway modulates FrzS dynamics. Although most cells still had the brighter fluorescent patch at the leading cell pole, pole-to-pole oscillations of FrzS were not observed in this mutant (Table 1). Rarely, a cell was observed to reverse its direction of movement, but then FrzS polar switching failed to occur (Table 1). These reversal events may have been caused by unregulated activity of the other motility motor, the A-motility system (see fig. S1). Some *frz* mutants (*frzCD*^{c}) show hyperreversals, hypothesized to be caused by constitutive signaling through the Frz pathway (*11*). FrzS-GFP expressing strains containing the *frzCD*^{c} mutation showed a ninefold increase in reversal frequency; these reversals were always accompanied by FrzS polar switching (Table 1). A fluorescence microscopy time-lapse series of a FrzS-GFP *frzCD*^{c} mutant cell is shown (Fig. 1C; movie S2). The cell pictured reversed its direction three times in the 10-min filming interval and showed corresponding oscillations in FrzS-GFP localization. The oscillatory period of FrzS-GFP was markedly reduced in the mutant, but the pattern was very similar to the one observed in the parent strain: Reversals always happened together with dispersal of the fluorescence at the old leading pole (after equalization of the fluorescent signals at both poles), followed by a rapid increase in fluorescence at the new leading pole. Additional nonpolarly localized FrzS clusters

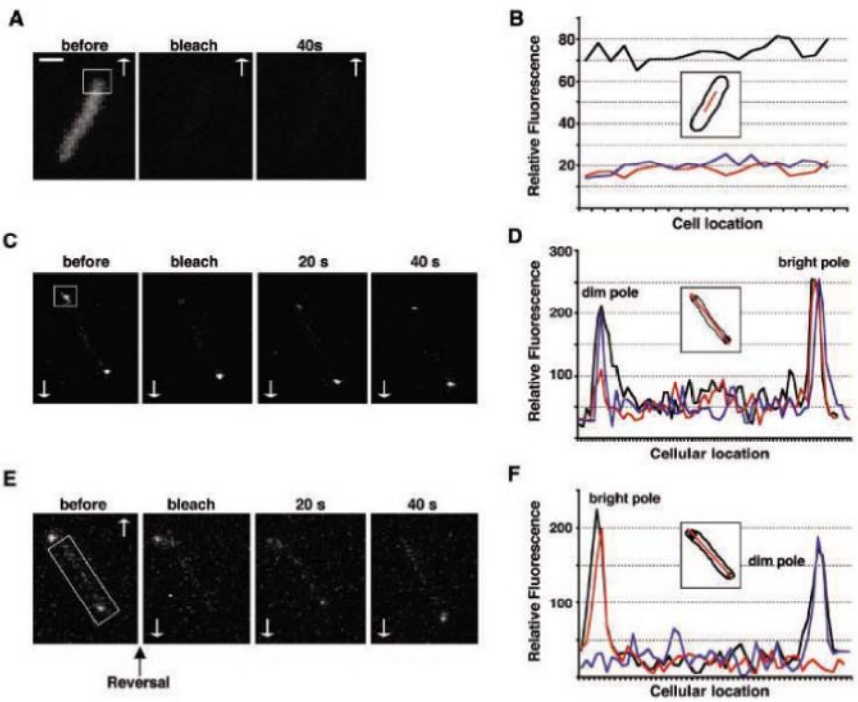

Fig. 2. FrzS-GFP dynamics in moving cells. (**A**) Diffusible GFP is fully bleached in the course of a 10-s laser exposure of the cell pole. Micrographs of a cell before bleaching, immediately after bleaching, and 40 s after bleaching. The open rectangle shows the area exposed to the laser. The white arrow shows the direction of movement. Scale bar, 2 μm. (**B**) Quantitative fluorescence analysis of the cell presented in (A) at different times. The relative fluorescence intensities (arbitrary units) were plotted as a function of the cellular location. Black line, fluorescence intensities before photobleaching; red line, fluorescence intensities immediately after photobleaching; blue line, fluorescence intensities 40 s after photobleaching. The inset represents the region of the cell that was selected (red line) to obtain the fluorescence intensity profile. (**C**) FRAP analysis of FrzS-GFP fluorescence at the dimmer pole. Labels as in (A). (**D**) Quantitative fluorescence analysis of the cell presented in (C) at different times. Labels as in (B). (**E**) FRAP analysis of FrzS-GFP fluorescence upon cellular reversal. Labels as in (A). (**F**) Quantitative fluorescence analysis of the cell presented in the (E) at different times. Labels as in (B).

were observed in 50% of the *frzCD*$^{+}$ cells; they faded and reappeared periodically (Fig. 1C; movie S2). The cluster closer to the new brighter pole always increased in intensity before the poles switched, which suggests that these foci represent fixed sites where FrzS could accumulate transiently as it moved from pole to pole. Thus, the periodicity of FrzS oscillations is controlled by the signaling activity of the Frz pathway.

The observed FrzS oscillations could not be attributed to targeted proteolysis followed by de novo protein synthesis because chloramphenicol (Cm) did not affect reversals or FrzS pole-to-pole switching. To investigate the velocity of FrzS-GFP movements in cells, we conducted a Fluorescence Recovery After Photobleaching (FRAP) experiment in the presence of Cm. (Fig. 2, A and B). Cells expressing diffusible GFP were illuminated in a small region for 10 s, which bleached the diffusible molecules throughout the cell (Fig. 2, A and B). When moving FrzS-GFP expressing cells were illuminated at only the dimmer cell pole for 10 s, the dimmer pole was bleached, but recovered almost full fluorescence after 40 s (Fig. 2, C and D). Thus, the movement of FrzS-GFP molecules was much slower than diffusible GFP, which suggests that it is not transported by diffusion. Moreover, fluorescence at the brighter pole was unaffected by the treatment and decreased only slightly after 40 s (Fig. 2, C and D). This suggests that the slow increase in fluorescence at the trailing pole observed before reversal (Fig. 1, B and C) was driven both by FrzS-GFP molecules that were not localized at the leading pole and FrzS-GFP molecules that came from the leading pole.

We showed that when cells reverse, the new leading pole increases in fluorescence; this increase peaked ~1 min after fluorescence dispersed at the old leading pole (Fig. 1, B and C). We used FRAP to test whether this increase was due to FrzS-GFP's leaving the old leading pole for the new leading pole. A moving cell was illuminated immediately before reversal such that only the leading pole would be unbleached. Fluorescence dispersed at the old leading pole and accumulated at the new leading pole; after 40 s most of the fluorescent signal had moved to the new leading pole (Fig. 2, E and F). Because fluorescence recovery at the new leading pole is essentially due to FrzS-GFP molecules leaving the old leading pole, we can estimate the speed of FrzS-GFP movement to be ≤ 0.3 μm/s (*12*).

In moving cells, cytosolic FrzS-GFP fluorescence could be detected as transient "comet tails" leading to the poles or as moving foci in some longer cells (see Fig. 1B; fig. S3). In many nonmoving cells, individual FrzS-GFP foci could be easily followed as they moved between cell poles (Fig. 3A; movie S3). Movement of the FrzS-GFP focus was slow: It took 5 min to travel the distance separating the two cell poles (Fig. 3A). The nonpolar fluorescence was detected as a patch, but also as a filament that overlapped the trajectory (Fig. 3B). An overlay of the time-lapse frames showed that the trajectory of the focus displayed two turns (Fig. 3B).

We also analyzed a stable mutant, $frzS_{\Delta 537\text{-}548}$, lacking a motif at the C-terminal end of FrzS required for polar localization (Fig. 3D). As expected, $FrzS_{\Delta 537\text{-}548}$ was unable to swarm, which indicates that polar localization is essential for function (Fig. 3C). Immunofluorescence staining of $FrzS_{\Delta 537\text{-}548}$ and deconvolution microscopy showed that FrzS organized as broad slanted bands and as clusters bordering the cell periphery (Fig. 3D). Although the pattern was not always continuous, volume reconstructions clearly showed that segments could be resolved as coiled filaments that run along the length of the cell (Fig. 3D). The localization pattern of the FrzS C-terminal variant could be due to self-organization, but it also suggests the presence of a filament that could explain the trajectory of moving FrzS-GFP foci. Indeed, $FrzS_{\Delta 537\text{-}548}$ may remain bound to the filament because it is unable either to track on the filament or to accumulate at the poles. The structures of ten $FrzS_{\Delta 537\text{-}548}$ filaments all showed turns (Fig. 3D), which suggests that these filaments could account for trajectories such as those observed (Fig. 3B).

The data presented show that S-motility reversals involve switching the sites of pili extrusion from one cell pole to the other. We hypothesize that a protein complex that contains FrzS tracks from pole to pole and controls pili assembly. Switching may be achieved by moving the complex along a cytoskeletal track (fig. S4). Bacterial cytoskeletal filaments have been shown to play an important role in the control of other cellular processes, such as cell division and DNA segregation (*13*).

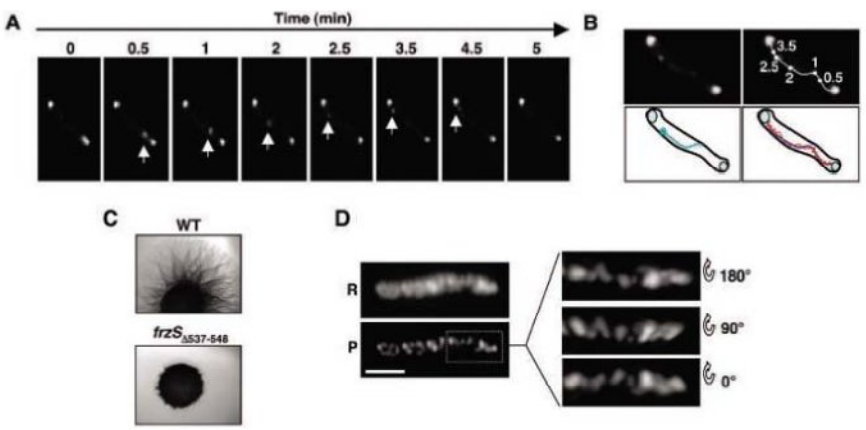

Fig. 3. FrzS may track along a helical filament. (**A**) Time-lapse fluorescence microscopy of a dynamic FrzS cluster. The cell was filmed for 5 min. White arrows point to the observed dynamic FrzS spot. (**B**) Trajectory of the moving complex. (Left) Enhanced view of the cell shown in (A) after 2.5 min (top) and schematics of the fluorescence signal (bottom). (Top right) The images shown in (A) were overlaid, and the spots observed at different times were linked to obtain a trajectory. The numbers refer to the times at which the foci were seen at a particular subcellular location. (Bottom right) Schematics of the trajectory (blue line) overlaid on the proposed coiled track (red line). (**C**) $FrzS_{\Delta 537\text{-}548}$ is defective for vegetative swarming. Motility phenotypes of the WT and $frzS_{\Delta 537\text{-}548}$ strains on S-motility–specific CYE-rich medium 0.3% agar plates (*11*). (**D**) Subcellular localization of $FrzS_{\Delta 537\text{-}548}$. The localization pattern of $FrzS_{\Delta 537\text{-}548}$ was determined by immunostaining using the FrzS-specific antiserum. R, raw image; P, processed image. (Right) Clockwise 90° rotations of the reconstructed volume of the segment boxed in the processed image. Scale bar, 2 μm.

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Supporting Online Material

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Retrograde Signaling by Syt 4 Induces Presynaptic Release and Synapse-Specific Growth

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The molecular pathways involved in retrograde signal transduction at synapses and the function of retrograde communication are poorly understood. Here, we demonstrate that postsynaptic calcium 2+ ion (Ca^{2-}) influx through glutamate receptors and subsequent postsynaptic vesicle fusion trigger a robust induction of presynaptic miniature release after high-frequency stimulation at *Drosophila* neuromuscular junctions. An isoform of the synaptotagmin family, synaptotagmin 4 (Syt 4), serves as a postsynaptic Ca^{2+} sensor to release retrograde signals that stimulate enhanced presynaptic function through activation of the cyclic adenosine monophosphate (cAMP)–cAMP-dependent protein kinase pathway. Postsynaptic Ca^{2} influx also stimulates local synaptic differentiation and growth through Syt 4–mediated retrograde signals in a synapse-specific manner.

Neuronal development requires coordinated signaling to orchestrate pre- and postsynaptic maturation of synaptic connections. Synapse-specific enhancement of synaptic strength as occurs during long-term potentiation (*1*), as well as compensatory homeostatic synaptic changes, have been suggested to require retrograde signals for their induction (*2*, *3*). Although retrograde signaling has been implicated widely in synaptic plasticity, the molecular mechanisms that transduce postsynaptic Ca^{2+} signals during enhanced synaptic activity to alterations in presynaptic function are poorly characterized. Because postsynaptic Ca^{2+} is essential for synapse-specific potentiation (*4*), it is important to characterize how Ca^{2+} can regulate retrograde communication at synapses.

To dissect the mechanisms underlying activity-dependent synaptic plasticity, we tested whether newly formed *Drosophila* glutamatergic neuromuscular junctions (NMJs), which have ~30 active zones, show physiological changes after 100-Hz stimulation (*5*). Within 1 min after stimulation, a gradual 100-fold increase in miniature excitatory postsynaptic current (miniature) frequency was observed (Fig. 1, A to C), from a baseline of 0.03 Hz to often more than 5 Hz. The high-frequency-stimulation-induced miniature release (termed HFMR) continued for a few minutes to as long as 20 min before subsiding to baseline levels. Perfusion of postsynaptic muscles with the Ca^{2-} chelator EGTA from the patch pipette caused a modest suppression of HFMR, whereas the fast Ca^{2} chelator 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) induced strong suppression by 2.5 min of perfusion. Longer perfusion with BAPTA for 5 min before stimulation abolished HFMR (Fig. 1, A to C), indicating HFMR is induced after postsynaptic Ca^{2-} influx.

Ca^{2-}–induced vesicle fusion in presynaptic terminals provides a temporally controlled and spatially restricted signal essential for synaptic communication. Postsynaptic vesicles within dendrites have been visualized by transmission electron microscopy (*6*), and dendritic release of several neuromodulators has been reported (*7*). To test whether postsynaptic vesicle fusion might underlie the Ca^{2}-dependent release of retrograde signals, we blocked postsynaptic vesicle recycling by using the dominant negative *shibire*^{ts1} mutation, which disrupts endocytosis at elevated temperatures (*8*). We expressed *shibire*^{ts1} specifically in postsynaptic muscles by driving a *UAS-shibire*^{ts1} transgene (*9*) with muscle-specific *myosin heavy chain* (*Mhc*)-*Gal4*, keeping presynaptic activity intact. At the permissive temperature (23°C), high-frequency stimulation induced normal HFMR (Fig. 1, D and F). However, raising the temperature to 31°C suppressed HFMR in the presence of postsynaptic *shibire*^{ts1}, whereas wild-type animals displayed normal HFMR at 31°C (Fig. 1, D and F). Basic synaptic properties in *Mhc-Gal4*, *UAS-shibire*^{ts1} animals were not affected at either the permissive or the restrictive temperature (Fig. 1G). The suppression of HFMR is not due to irreversible damage induced by postsynaptic *UAS-shibire*^{ts1} expression, because a second high-frequency stimulation after recovery to the permissive temperature induced normal HFMR (Fig. 1, E and H).

The synaptic vesicle protein synaptotagmin 1 (Syt 1) is the major Ca^{2+} sensor for vesicle fusion at presynaptic terminals (*10*, *11*) but is not localized postsynaptically. We have recently shown that another isoform of the synaptotagmin family, synaptotagmin 4 (Syt 4), is present in the postsynaptic compartment (*12*), suggesting Syt 4 might function as a postsynaptic Ca^{2} sensor. Syt 4 immunoreactivity is observed in a punctate pattern surrounding presynaptic terminals, suggesting Syt 4 is present on postsynaptic vesicles (Fig. 2B). We again blocked postsynaptic vesicle recycling by using the *UAS-shibire*^{ts1} transgene driven with *Mhc-Gal4*. Without a temperature shift, Syt 4–containing vesicles showed their normal postsynaptic distribution surrounding presynaptic terminals (Fig. 2B). When the temperature was shifted to 37°C for 10 min in the presence of high-K^{+} saline containing 1.5 mM Ca^{2} to drive synaptic activity, Syt 4–containing vesicles translocated to the plasma membrane (Fig. 2C). After recovery at 18°C for 20 min, postsynaptic vesicles returned to their normal position (Fig. 2D). Removing extracellular Ca^{2+} during the high-K stimulation resulted in vesicles that did not translocate to the postsynaptic membrane (Fig. 2E).

To further test whether the Syt 4 vesicle population undergoes fusion with the postsynaptic membrane as opposed to mediating fusion between intracellular compartments, we constructed transgenic animals expressing a pH-sensitive green fluorescent protein (GFP) variant (ecliptic pHluorin) (*13*) fused at the intravesicular N terminus of Syt 4. Ecliptic pHluorin increases its fluorescence 20-fold when exposed to the extracellular space from the acidic lumen of intracellular vesicles during fusion. Expression of Syt 4-pHluorin in postsynaptic muscles resulted in intense fluorescence at specific subdomains in the postsynaptic membrane, defining regions where Syt 4 vesicles undergo exocytosis (Fig. 2F). The fluorescence was not diffusely present over the postsynaptic membrane but directed to restricted compartments. We co-stained *Mhc-Gal4, UAS Syt 4 pHluorin* larvae with antibodies against the postsynaptic density protein, DPAK, and nc82, a monoclonal antibody against a presynaptic active zone protein (*5*). Syt 4 pHluorin colocalized with DPAK and localized adjacent to nc82, demonstrating that Syt 4 pHluorin translocates from postsynaptic vesicles to the plasma membrane at postsynaptic densities opposite presynaptic active zones (Fig. 2, G and H).

To examine the function of Syt 4–dependent postsynaptic vesicle fusion, we characterized the phenotype of a *syt 4* null mutant (*syt 4*^{RA1}) (*12*) and a *syt 4* deficiency (*rn*^{16}) (*14*). Mutants lacking Syt 4 hatch from the egg case 21 hours after egg laying at 25°C, similar to wild type, and grow to fully mature larvae that pupate and eclose with a normal time course. To determine whether postsynaptic vesicle fusion

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triggered by Ca^{2+} influx is required for IIFMR, we analyzed the effects of high-frequency stimulation in *syt 4* mutants. In contrast to controls (Fig. 3A), the increase of miniature release was eliminated in *syt 4* mutants (Fig. 3B). Postsynaptic expression of a *UAS syt 4* transgene (*15*) completely restored IIFMR in the null mutant (Fig. 3C), demonstrating that postsynaptic Syt 4 is required for triggering enhanced presynaptic function. Presynaptic expression of a *UAS–syt 4* transgene did not restore IIFMR (Fig. 3E). In addition, postsynaptic expression of a mutant Syt 4 with neutralized Ca^{2+}-binding sites in both C2A and C2B domains did not rescue IIFMR, indicating that retrograde signaling by Syt 4 requires Ca^{2+} binding (Fig. 3D).

The large increase in miniature frequency observed during IIFMR is similar to the enhancement of presynaptic release after activation of cyclic adenosine monophosphate (cAMP) dependent protein kinase (PKA) described in *Aplysia* (*2*) and *Drosophila* (*16*). Bath application of forskolin, an activator of adenylyl cyclase, results in a robust enhancement of miniature frequency at *Drosophila* NMJs (Fig. 3G) similar to that observed during HFMR, suggesting retrograde signals may function to increase presynaptic cAMP. To test the role of the cAMP-PKA pathway in IIFMR, we assayed *DC0* mutants (*17*) for the presence of HFMR. *DC0* encodes the major catalytic subunit of PKA in *Drosophila* and has been implicated in olfactory learning (*18*). Similar to the lack of forskolin-induced miniature induction (*19*), *DC0* null mutants lacked

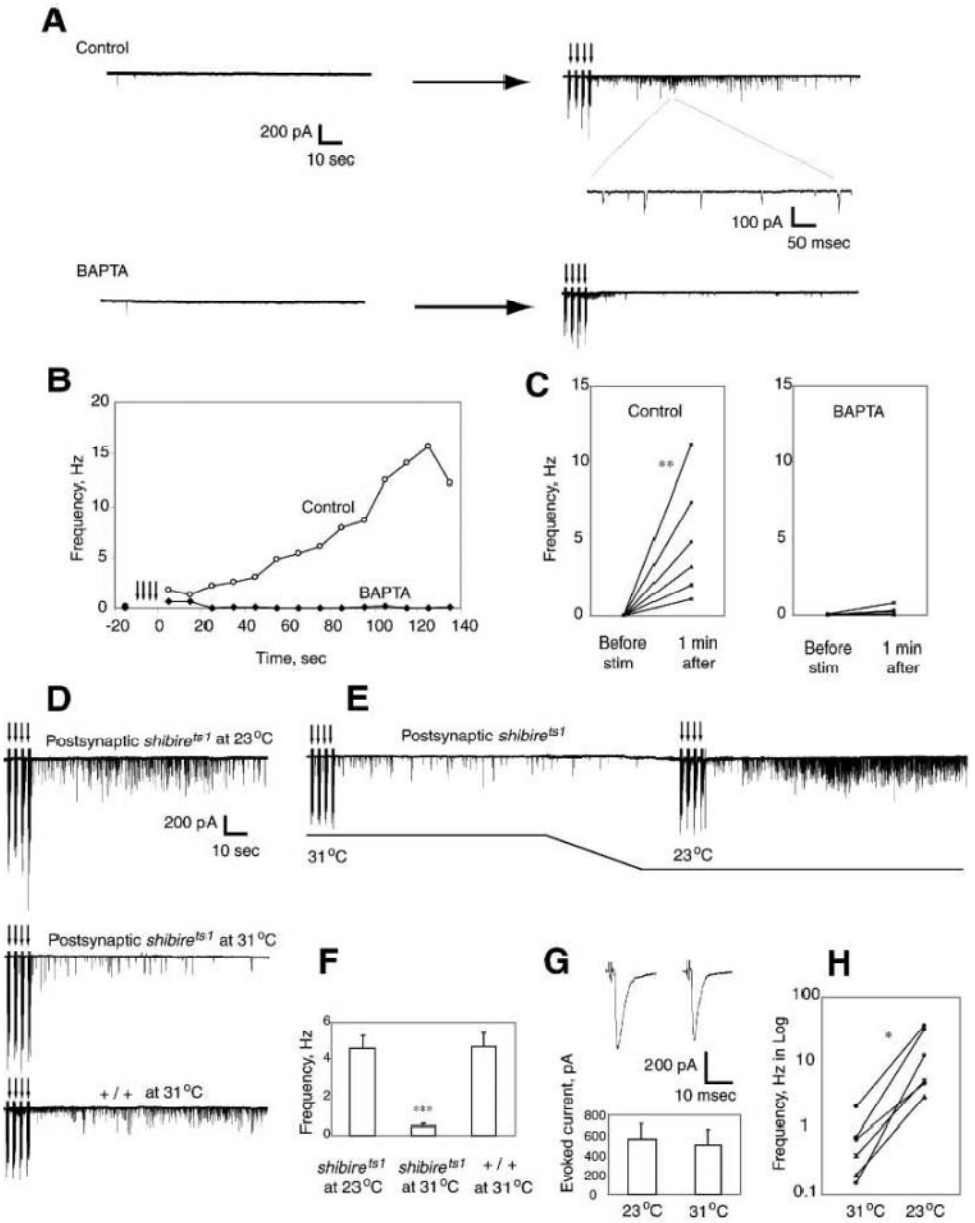

Fig. 1. High-frequency stimulation triggers enhanced presynaptic miniature release that requires postsynaptic Ca^{2+} and postsynaptic vesicle trafficking. (**A**) A motor nerve innervating embryonic muscle fiber 6 at hatching stage (21 hours after egg laying) was stimulated with four trains of 1-s 100-Hz stimuli spaced 2 s apart in 0.5 mM extracellular Ca^{2-}. Whereas spontaneous release is rarely seen without stimulation (top left), high-frequency stimulation (represented by arrows) induces a 100-fold increase in frequency of miniatures (top right). The lower image shows traces when BAPTA (5 mM) was included in the internal solution of the patch electrode. (**B**) Representative time courses of HFMR from control and BAPTA-treated muscle cells. At each 10-s interval, miniatures are displayed as mean frequency. The first time point represents averaged miniature frequency for 5 min before stimulation. (**C**) Miniature frequency at 1 min after 100-Hz stimulation (calculated mean between 50 and 70 s after stimulation), compared with miniature frequency before stimulation (mean for 5 min before stimulation). The number of samples analyzed were six for control and five for BAPTA perfusion. Double asterisks indicate $P < 0.01$ by paired *t* test. (**D**) High-frequency stimulation performed in *Mhc-Gal4, UAS-shibire^{ts1}* animals at permissive (23°C) and restrictive (31°C) temperatures. Wild-type animals show normal HFMR at 31°C (bottom). (**E**) Temperature shift experiments from the restrictive (31°C) temperature to the permissive (23°C) temperature using *Mhc-Gal4, UAS-shibire^{ts1}* animals. The scale in the top of (**D**) also applies to the middle and bottom of (D) and (E). (**F**) Quantification of miniature frequency at 1 min after high-frequency stimulation calculated as described above. Triple asterisks indicate *Mhc-Gal4, UAS-shibire^{ts1}* at 31°C is significantly different both from *Mhc-Gal4, UAS-shibire^{ts1}* at 23°C and from wild type at 31°C with the use of posthoc comparisons (Scheffe's multiple comparisons test; $P < 0.001$) after single-factor analysis of variance. The number of samples analyzed were six for *Mhc-Gal4, UAS-shibire^{ts1}* at 23°C, 13 for *Mhc-Gal4, UAS-shibire^{ts1}* at 31°C, and four for wild type at 31°C. (**G**) Evoked responses in *Mhc-Gal4, UAS-shibire^{ts1}* animals at the permissive and restrictive temperatures. The numbers of samples analyzed were six at 23°C and six at 31°C. Error bars in (F) and (G) indicate SEM. (**H**) Miniature frequency in the temperature shift experiments (E) at 1 min after high-frequency stimulation calculated as described above. The number of samples analyzed was six. The asterisk indicates $P < 0.05$ (Wilcoxon paired-sample test).

HFMR (Fig. 3F). Bath application of forskolin in *syt 4* mutants resulted in enhanced miniature frequency (Fig. 3H), suggesting activation of the cAMP pathway can bypass the requirement for Syt 4 in synaptic potentiation.

To further explore the role of retrograde signaling at *Drosophila* synapses, we characterized the role of activity in synapse differentiation and growth. During *Drosophila* embryonic development, presynaptic terminals undergo a stereotypical structural change from a flat path-finding growth cone into varicose synaptic terminals through dynamic reconstruction (*20*). Such developmental changes in synaptic structure may share common molecular mechanisms with morphological changes induced during activity-dependent plasticity. We eliminated synaptic transmission by using a deletion mutation that removes the postsynaptic glutamate receptors, DGluRIIA and DGluRIIB (*21*) (hereafter referred to as GluRs). Postsynaptic currents normally induced by nerve stimulation were completely absent in the mutants (*gluR*) (fig. S1, A and B). Miniatures were also eliminated, even at elevated extracellular Ca^{2+} concentrations of 4 mM. In the absence of GluRs, the presynaptic morphology of motor terminals is abnormal, even though GluRs are only expressed in postsynaptic muscles (*22*). GluR-deficient terminals maintain a flattened growth cone–like structure and fail to constrict into normal synaptic varicosities (Fig. 4, A and B, and fig. S1, C and D; see fig. S1N for quantification). We also assayed synaptic development in a null mutant of the presynaptic plasma membrane t-SNARE [SNAP (soluble *N*-ethylmaleimide–sensitive factor attachment protein) receptor], syntaxin (*syx*), which eliminates neurotransmitter release (*23*), providing an inactive synapse similar to that in the *gluR* mutant. *syx* null mutants also have abnormal growth cone–like presynaptic terminals with less varicose structure (fig. S1E).

Because activity is required for synapse development, we tested whether Syt 4–dependent vesicle fusion may be required, similar to its role in acute retrograde signaling during HFMR. Physiological analysis revealed that the amplitude of evoked currents in mutants lacking Syt 4 was moderately reduced compared with wild type (fig. S1, F and G), suggesting weaker synaptic function or development. Similar to the morphological phenotype of the *gluR* mutant (Fig. 4B and fig. S1, D and N), *syt 4* null mutant embryos showed defective presynaptic differentiation (Fig. 4C and fig. S1, H and N). Nerve terminals lacking Syt 4 displayed reduced varicose structure, whereas wild-type terminals have already formed individual varicosities at this stage of development (Fig. 4A and fig. S1, C and N). Postsynaptic expression with a *UAS syt 4* transgene rescued the physiological and morphological phenotypes (fig. S1, F, G, I, and N). Syt 4 Ca^{2+}-binding deficient mutant transgenes did not rescue either the morphological immaturity or the reduced amplitude of evoked currents (fig. S1, F, G, I, and N), even though Syt 4 immunoreactivity at the postsynaptic compartment was restored by muscle-specific expression of the mutant *syt 4* transgene (fig. S1M), similar to the wild-type *syt 4* transgene (fig. S1L) and endogenous Syt 4 (Fig. 2B) immunoreactivity.

Mammalian *syt 4* was originally identified as an immediate-early gene that is transcriptionally up-regulated by nerve activity in certain brain regions (*24*). We therefore analyzed gain-of-function phenotypes caused by postsynaptic Syt 4 overexpression specifically in muscle cells to increase the probability of postsynaptic vesicle fusion. Syt 4 overexpression induced overgrowth of presynaptic terminals in mature third instar larvae (fig. S2, A

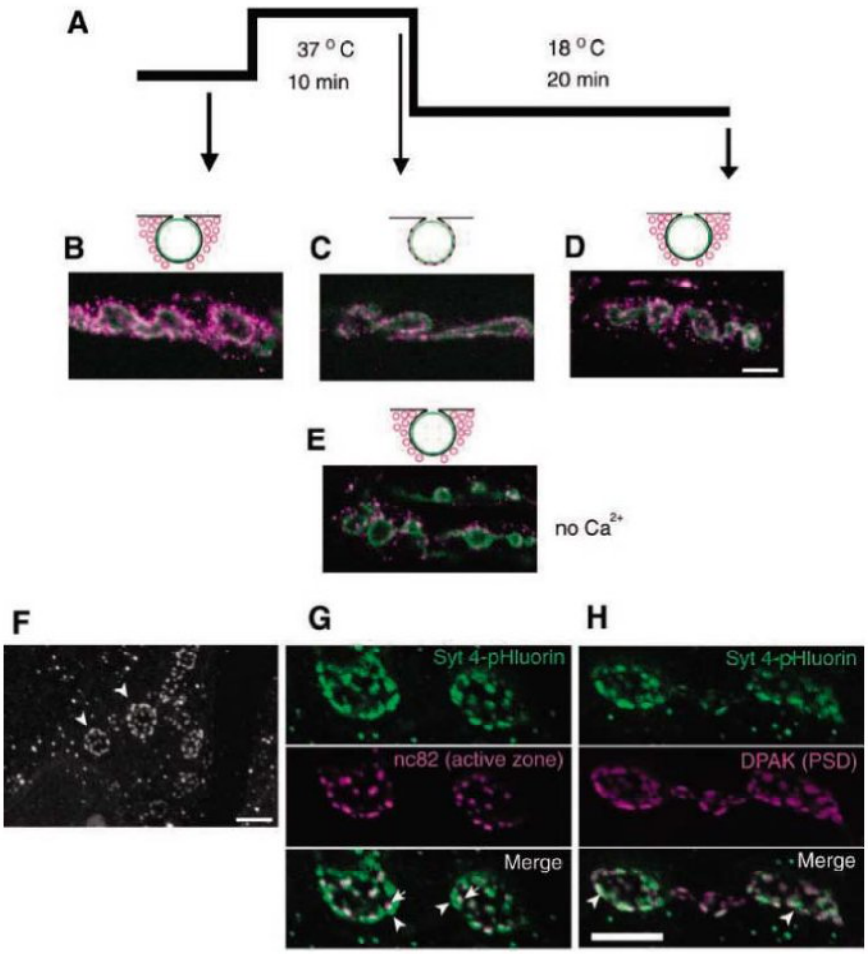

Fig. 2. Ca^{2+}-dependent translocation of Syt 4–positive vesicles to the postsynaptic membrane. (**A** to **E**) *Mhc-Gal4, UAS-shibire^{ts1}* third instar larval NMJs were stained with antisera against Syt 4 (magenta) to visualize postsynaptic vesicles and anti-horseradish peroxidase (HRP) (green) to visualize the presynaptic plasma membrane. (A) Diagram of temperature shifts performed. (B) Larvae maintained at 21°C have abundant postsynaptic vesicles that form a cloud around presynaptic terminals. (C) Larvae shifted to 37°C for 10 min in high-K^+ saline (60 mM K^+ and 1.5 mM Ca^{2+}) lose the Syt 4–positive vesicle halo surrounding synaptic terminals, with a shift in the signal to the muscle plasma membrane. (D) Recovery of the Syt 4 vesicle population was observed after lowering the temperature to 18°C for 20 min. (E) Temperature shifts in 60 mM K^+ saline without Ca^{2+} resulted in no Syt 4 vesicle translocation. The scale bar in (D) is 5 μm and applies to all images in (B) to (E). Above each image are schematic diagrams depicting the distribution of Syt 4 signal at each time point. (**F** and **H**) *Mhc-Gal4, UAS–Syt 4–pHluorin* localizes to the postsynaptic density. (F) Live images of Syt 4–pHluorin by confocal microscopy at the third instar larval NMJ, showing a projection of optical sections through the Z axis (Fig. 4C). Arrowheads point to varicosities with patches of Syt 4–pHluorin signal. Scale bar is 5 μm. (G) A Syt 4–pHluorin–expressing muscle (green, upper panel) costained with nc82 (magenta, middle panel) in an optical section of a fixed third instar NMJ. Syt 4–pHluorin–positive patches (arrowheads) are localized adjacent to presynaptic active zones (arrows). (H) Syt 4–pHluorin (green, top) colocalized with immunoreactivity against DPAK (magenta, middle; arrowheads). The scale bar in (H) is 5 μm and also applies to (G).

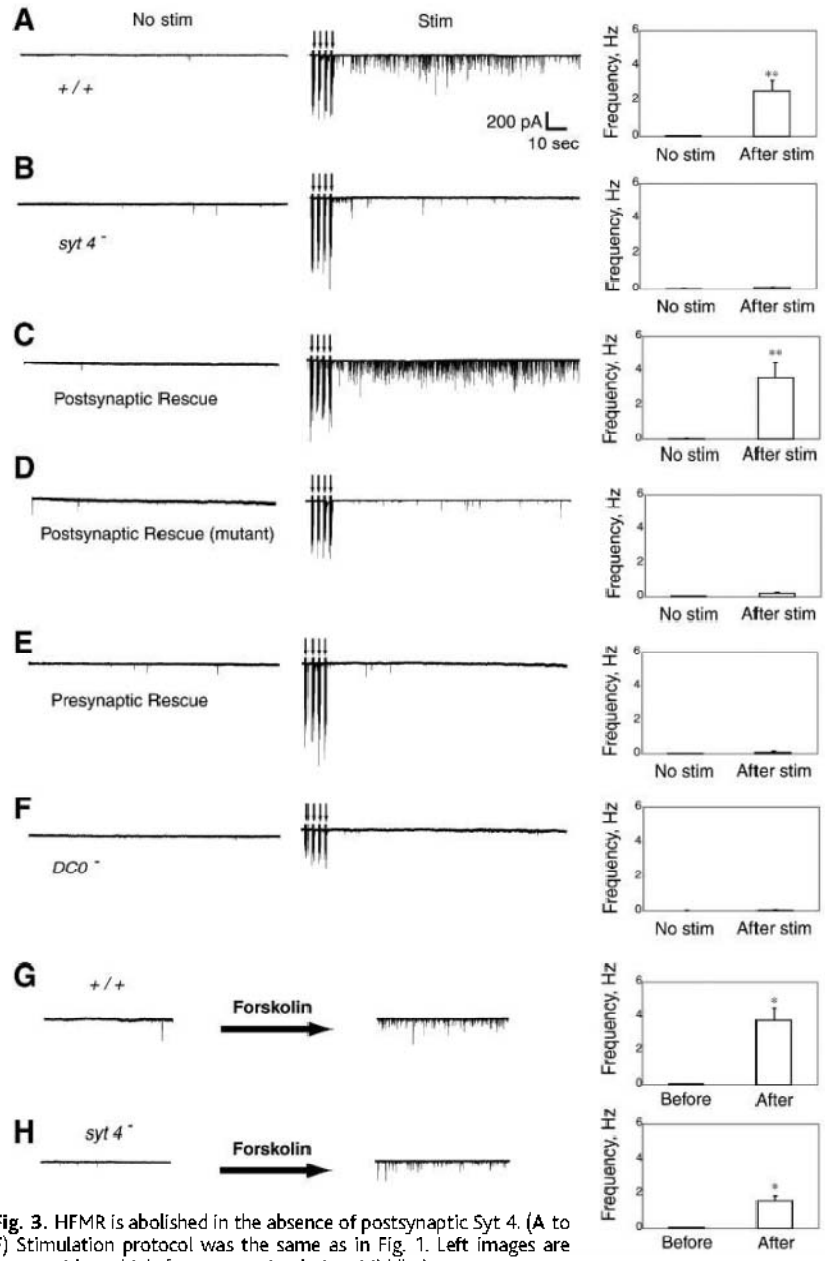

Fig. 3. HFMR is abolished in the absence of postsynaptic Syt 4. (**A** to **F**) Stimulation protocol was the same as in Fig. 1. Left images are traces without high-frequency stimulation. Middle images are representative traces when stimulated. Whereas spontaneous release is rarely seen without stimulation [left in (A)], high-frequency stimulation induces a robust HFMR response [middle in (A)]. The induction of presynaptic miniature release is abolished in the *syt 4* null mutant [*syt* 4^{BA1} (B); rn^{16} showed an indistinguishable phenotype] but restored in postsynaptically rescued synapses by *Mhc-Gal4, UAS–syt 4* [rn^{16} background (C)]. A *syt 4* transgene with mutations in the C2A and C2B Ca^{2+}-binding sites, *UAS–syt 4 [C2A D4N, C2B D3,4N]*, did not rescue HFMR in the null mutant [*syt* $4^{BA1}/rn^{16}$ background (D)]. Presynaptic expression by *elav-Gal4, UAS–syt 4* did not rescue the loss of HFMR (E). HFMR was not observed in a null mutant of PKA, $DC0^{B3}$ (F). (Right graphs) Miniature frequency at 1 min after tetanic stimulation compared with miniature frequency without stimulation (mean for 2 min). Number of samples analyzed: (A) nine no stimulation and eight stimulation, (B) nine no stimulation and six stimulation, (C) five no stimulation and five stimulation, (D) 10 no stimulation and six simulation, (E) seven no stimulation and five stimulation, and (F) five no stimulation and five stimulation. Double asterisks indicate $P < 0.01$ by Mann-Whitney's U test. Error bars are SEM. (**G** and **H**) Left images show representative traces of recordings before application of forskolin (500 μM) to activate PKA, and middle images show traces at 20 min after application. Both wild type (G) and the *syt 4* null mutant [*syt* 4^{BA1} (H)] showed 100-fold increases in miniature release. (Right graphs) Miniature frequency before stimulation and 20 min after application of forskolin. Number of samples analyzed: (G) four, before and after; (H) four, before and after. Single asterisks indicate $P < 0.05$ by paired *t* test. Error bars are SEM. The scale for trace in (A) applies to all traces in (A) to (H).

to C), in contrast to overexpression of Syt 1 (fig. S2C), which does not traffic to Syt 4 containing postsynaptic vesicles (*25*). In addition to synaptic overgrowth, Syt 4 overexpression occasionally induced the formation of abnormally large varicosities. Postsynaptic overexpression of the Syt 4 Ca^{2+}-binding mutant did not induce synaptic overgrowth (fig. S2C), indicating that retrograde signaling by Syt 4 also requires Ca^{2+} binding to promote synaptic growth.

To determine whether the cAMP-PKA pathway is important in activity-dependent synaptic growth, we assayed the effects of PKA on synaptic morphology. Expression of constitutively active PKA (*26*) presynaptically using a motor neuron specific *Gal4* driver induced not only synaptic overgrowth but also larger individual varicosities in mature third instar larvae (fig. S2D), similar to those induced by postsynaptic overexpression of Syt 4. These observations are consistent with the presynaptic overgrowth observed in the learning mutant, *dunce*, which disrupts the enzyme that degrades cAMP (*27*), and with studies in *Aplysia* implicating PKA in synaptic varicosity formation (*2*). We next characterized the loss-of-function phenotype of PKA mutants ($DC0^{B3}$) (*17*) at the embryonic NMJ (Fig. 4D) to compare with *gluR* and *syt 4* mutants (Fig. 4, B and C). Presynaptic terminals in the *DC0* mutant were morphologically aberrant, with abnormal growth cone like features and less varicose structure. Postsynaptic expression of a constitutively active PKA transgene in the *DC0* or *syt 4* mutant backgrounds rescued the immature morphology (Fig. 4, E and F), suggesting activation of PKA is downstream of Syt 4 dependent release of retrograde signals.

Similar to the role of Syt 1 dependent synaptic vesicle fusion in triggering synaptic transmission at individual synapses, Syt 4–dependent vesicle fusion might trigger synapse-specific plasticity and growth. To test synapse specificity, we took advantage of the specific properties of the *Drosophila* NMJ at muscle fibers 6 and 7, where two motorneurons innervate both muscle fibers 6 and 7 during development (Fig. 4G). We expressed Syt 4 specifically in embryonic muscle fiber 6 but not muscle fiber 7 by using the *1194-Gal4* driver (*28*). If Syt 4–dependent retrograde signals induce general growth of the motorneuron, one would expect to see a proliferation of synapses on both muscle fibers. Alternatively, if Syt 4 promoted local synaptic growth, one would expect specific activation of synapse proliferation only on target muscle 6, releasing the Syt 4 dependent signal. *UAS syt 4* driven by *H94-Gal4* increased innervation on muscle fiber 6 compared with that on muscle fiber 7 in third instar larvae (fig. S2, F to H). Control experiments with Syt 4 Ca^{2+}-binding deficient mutant transgenes, or a

transgene encoding Syt 1, did not result in proliferation (fig. S2, E, G, and H). Thus, synaptic growth can be preferentially directed to specific postsynaptic targets where Syt 4 dependent retrograde signals predominate, allowing differential strengthening of active synapses via local rewiring.

On the basis of the results described above, we propose a local feedback model for activity-dependent synaptic plasticity and growth at *Drosophila* NMJs (Fig. 4H). Synapse-specific Ca^{2+} influx triggers postsynaptic vesicle fusion through Syt 4. Fusion of Syt 4 containing vesicles with the postsynaptic membrane releases locally acting retrograde signals that activate the presynaptic terminal, likely through the cAMP pathway. Active PKA then triggers cytoskeletal changes by unknown effectors to induce presynaptic growth and differentiation. Moreover, PKA is well known to facilitate neurotransmitter release directly, triggering a local synaptic enhancement of presynaptic release as shown in HFMR. Therefore, postsynaptic vesicular fusion might initiate a positive feedback loop, providing a localized activated synaptic state that can be maintained beyond the initial trigger.

As a general mechanism for memory storage, Hebb postulated that potentiated synapses maintain an activated state until structural changes occur to consolidate alterations in synaptic strength (*29*). Our results demonstrate that acute plasticity and synapse-specific growth require Syt 4–dependent retrograde signaling at *Drosophila* NMJs. The feedback mechanism described here could be a molecular basis for both input-specific postsynaptic tagging (*30*) and an output-specific presynaptic mark or tag (*31*) for long-lasting potentiation. The regenerative nature of a positive feedback signal allows individual synapses to be tagged in a discrete all-or-none manner until synaptic rewiring is completed. The synaptic tag is maintained as a large increase in miniature frequency at *Drosophila* NMJs, suggesting a previously unknown role for miniature release in neuronal function. The spatial resolution for input and output specificity would result from the accuracy insured by Ca^{2+}-dependent vesicle fusion and subsequent diffusion, similar to the precision of presynaptic neurotransmitter release.

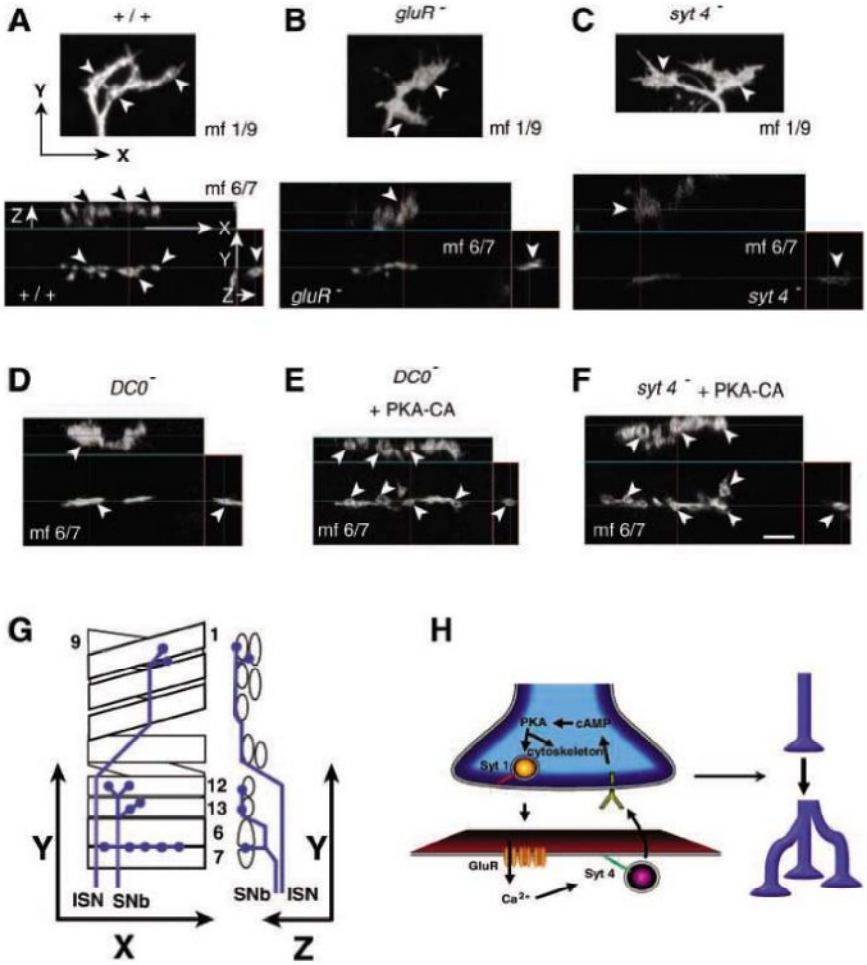

Fig. 4. Postsynaptic activity–dependent presynaptic development mediated through retrograde signaling by Syt 4 and presynaptic PKA. (**A** to **F**) Presynaptic morphology of embryonic NMJs at hatching stage (21 hours after egg laying). Presynaptic terminals innervating muscle fibers 1 and 9 [top images in (A) to (C)] and muscle fibers 6 and 7 [bottom images in (A) to (C) and (D) to (F)] were stained with the neural membrane marker anti-HRP. Confocal micrographs in top images in (A) to (C) are shown as stacked images parallel to the body wall (*X-Y*), projected along the *Z* axis (perpendicular to the body wall), as indicated in (G). In bottom images in (A) to (C) and (D) to (F), confocal micrographs are shown in three axes, parallel to the body wall (*X-Y*), perpendicular to the body wall and longitudinal (*X-Z*), and perpendicular to the body wall and across the longitudinal axis (*Y-Z*), as indicated in (G). Wild-type embryos (A) have synaptic terminals that have constricted into individual varicosities (arrowheads). In *gluR* (B), the *syt 4* null mutant [$syt\ 4^{BA1}$ (C); rn^{16} showed an indistinguishable phenotype] and the null mutant of PKA, $DC0^{B3}$ (D), mutant terminals fail to constrict into normal varicosities and maintain a flat less-varicose appearance (arrowheads) spreading through the *X-Y* plane or through the *X-Z* plane. Presynaptic expression of a constitutively active PKA transgene using the motor neuron–specific driver, *D42-Gal4*, restored normally constricted varicosities (arrowheads) in the $DC0^{B3}$ background (E) and in the $syt\ 4^{BA1}$ background (F). The scale bar in (F) is 5 μm and applies to all images in (A) to (F). (G) Schematic diagrams to show the arrangement of muscles (labeled 1, 9, 12, 13, 6, and 7) and motor terminals on the body wall in a *Drosophila* embryo. (H) Schematic diagram of a proposed local feedback model to describe synaptic plasticity and growth at *Drosophila* NMJs.

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www.sciencemag.org/cgi/content/full/310/5749/858/DC1
Materials and Methods
Figs. S1 and S2
References and Notes

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Fast Readout of Object Identity from Macaque Inferior Temporal Cortex

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Understanding the brain computations leading to object recognition requires quantitative characterization of the information represented in inferior temporal (IT) cortex. We used a biologically plausible, classifier-based readout technique to investigate the neural coding of selectivity and invariance at the IT population level. The activity of small neuronal populations (~100 randomly selected cells) over very short time intervals (as small as 12.5 milliseconds) contained unexpectedly accurate and robust information about both object "identity" and "category." This information generalized over a range of object positions and scales, even for novel objects. Coarse information about position and scale could also be read out from the same population.

Primates can recognize and categorize objects as quickly as 200 ms after stimulus onset (*1*). This remarkable ability underscores the high speed and efficiency of the object recognition computations by the ventral visual pathway (*2–5*). Because the feed-forward part of this circuitry requires at least eight or more synapses from the retina to anterior IT cortex, it has been proposed that the computations at each stage are based on just one or very few spikes per neuron (*6, 7*). At the end of the ventral stream, single cells in IT cortex show selectivity for complex objects with some tolerance to changes in object scale and position (*2–4, 6, 8–16*). Small groups of neurons in IT cortex tuned to different objects and object parts might thus provide sufficient information for several visual recognition tasks, including identification, categorization, etc. This information could then be "read out" by circuits receiving input from IT neurons (*17–19*).

Although physiological and functional imaging data suggest that visual object identity and category are coded in the activity of IT neurons (*2–6, 8–16, 20*), fundamental aspects of this code remain under debate, including the discriminative power in relation to population size, temporal resolution, and time course. These questions must be understood at the population level to provide quantitative constraints for models of visual object recognition. We examined these issues by obtaining independent recordings from a large unbiased sample of IT neuronal sites and using a population readout technique based on classifiers. The readout approach consists of training a regularization classifier (*21*) to learn the map from neuronal responses to each object label (Supporting Online Material), as in recent studies in the motor system [e.g., (*22*)]. Instead of making strong assumptions about the prior probability distribution of the training examples, the classifier learns directly from them and generalizes to novel responses (*21*). The input consists of the neuronal responses from the independently recorded neurons; different input representations allow quantitative comparisons among neural coding alternatives (*10, 13, 22–28*). After training, the classifier can be used to decode the responses to novel stimuli. We used a one-versus-all approach whereby for each class of stimuli (8 classes for categorization, 77 classes for identification, 3 classes for scale and position readout; see below), one binary classifier was trained. The overall classifier prediction on test data was given by the binary classifier with the maximum activation. The performance of such classifiers constitutes a lower bound on the information available in the population activity, but is a meaningful measure that could be directly implemented by neuronal hardware.

We used the classifier approach to determine the ability of more than 300 sequentially collected IT sites from two passively fixating monkeys to "categorize" 77 gray-scale objects as belonging to one of eight possible groups (*29*) (Fig. 1A). Figure 1B (red curve) shows the cross-validated performance of classifiers in performing this categorization task as a function of the number of recording sites (*30*). The spiking activity of 256 randomly selected multi-unit activity (MUA) sites was sufficient to categorize the objects with 94 ± 4% accuracy (mean ± SD; for 100 sites, interpolated performance = 81%; chance = 12.5%). Similarly, we tested the ability of the IT population to identify each of the 77 objects (Fig. 1B, blue curve). Even small populations of IT neurons were capable of performing this identification task at high accuracy (for 256 sites, 72 ± 3% correct; for 100 sites, interpolated performance = 49%; chance = 1.3%), although at lower performance than categorization for the same number of sites (*31*). Classifier performance increased approximately linearly with the logarithm of the number of sites, which is indicative of a distributed representation in contrast to a grandmother-like representation (*13, 28, 32, 33*). Very similar levels of performance were obtained when single unit activity (SUA) was considered [Fig. 1C, (*28*)]. The local field potentials also contain information about object category [Fig. 1C, (*28*)]. Examination of the classification errors suggests that some objects and categories were easier to discriminate than others (Fig. 1D). All the results reported here were obtained using a linear (regularized) classifier. Classification performance was similar for several different types of classifiers, and the performance of linear classifiers among the simplest classifiers could not be substantially improved upon (*28, 34*).

The performance values in Fig. 1, B to D, are based on the responses of single stimulus presentations that were not included in the classifier training. Thus, the level of recognition performance is what real downstream neurons could, in theory, perform on a single trial by simply computing a weighted sum of

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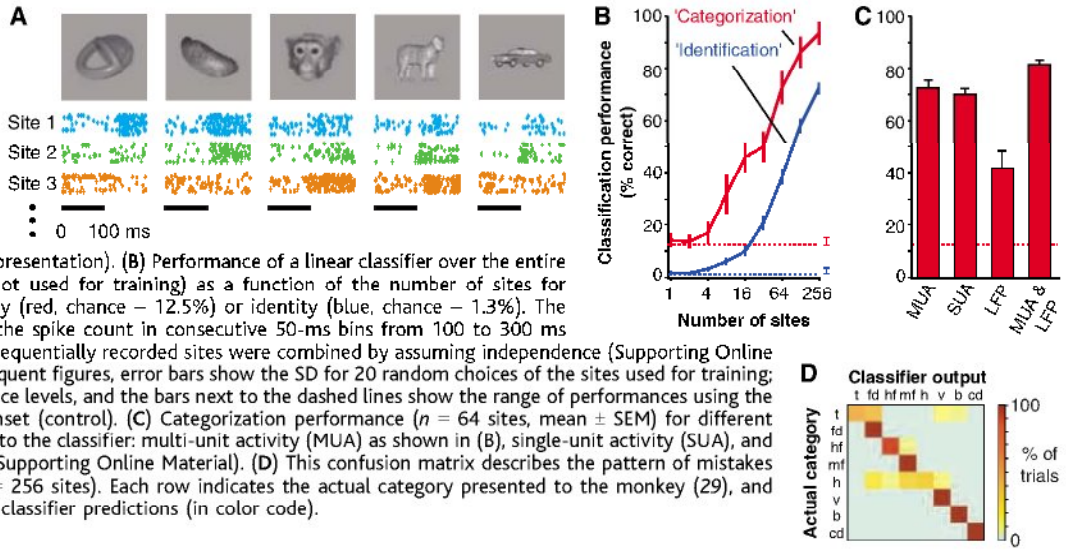

Fig. 1. Accurate readout of object category and identity from IT population activity. **(A)** Example of multi-unit spiking responses of 3 independently recorded sites to 5 of the 77 objects. Rasters show spikes in the 200 ms after stimulus onset for 10 repetitions (black bars indicate object presentation). **(B)** Performance of a linear classifier over the entire object set on test data (not used for training) as a function of the number of sites for reading out object category (red, chance = 12.5%) or identity (blue, chance = 1.3%). The input from each site was the spike count in consecutive 50-ms bins from 100 to 300 ms after stimulus onset (*28*). Sequentially recorded sites were combined by assuming independence (Supporting Online Material). In this and subsequent figures, error bars show the SD for 20 random choices of the sites used for training; the dashed lines show chance levels, and the bars next to the dashed lines show the range of performances using the 200 ms before stimulus onset (control). **(C)** Categorization performance (n = 64 sites, mean ± SEM) for different data sources used as input to the classifier: multi-unit activity (MUA) as shown in (B), single-unit activity (SUA), and local field potentials (LFP, Supporting Online Material). **(D)** This confusion matrix describes the pattern of mistakes made by the classifier (n = 256 sites). Each row indicates the actual category presented to the monkey (*29*), and each column indicates the classifier predictions (in color code).

spikes over a short time interval (100- to 300-ms interval divided into bins of 50 ms in this case) (*11*, *23*, *24*, *28*). This is notable considering the high trial-to-trial variability of cortical neurons (*27*). The IT population performance is also robust to biological noise sources such as neuronal death and failures in neurotransmitter release [fig. S1, (*35*)]. Although Fig. 1 (and most other decoding studies) assumes precise knowledge about stimulus onset time, this is not a limitation because we could also accurately read out stimulus onset time from the same IT population [fig. S5, (*28*)].

A key computational difficulty of object recognition is that it requires both selectivity (different responses to distinct objects such as one face versus another face) and invariance to image transformations (similar responses to, e.g., rotations or translations of the same face) (*8*, *12*, *17*). The main achievement of mammalian vision, and one reason why it is still so much better than computer vision algorithms, is the combination of high selectivity and robust invariance. The results in Fig. 1 demonstrate selectivity; the IT population can also support generalization over objects within predefined categories, suggesting that neuronal responses within a category are similar (*36*). We also explored the ability of the IT population to generalize recognition over changes in position and scale by testing 71 additional sites with the original 77 images and four transformations in position or scale. We could reliably classify (with less than 10% reduction in performance) the objects across these transformations even though the classifier only "saw" each object at one particular scale and position during training (Fig. 2). The "identification" performance also robustly generalized across position and scale (*28*). Neurons also showed scale and position invariance for novel objects not seen before (fig. S6). The IT population

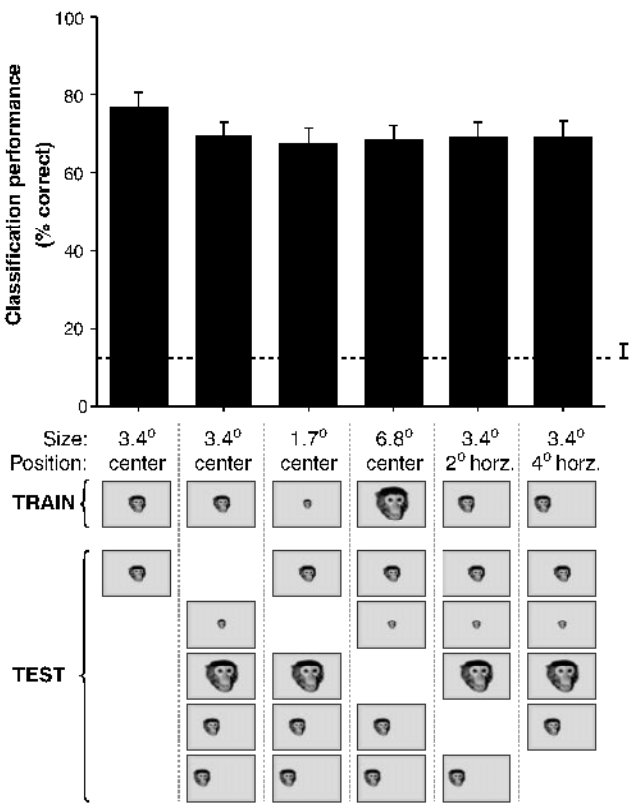

Fig. 2. Invariance to scale and position changes. Classification performance (categorization, n = 64 sites, chance = 12.5%) when the classifier was trained on the responses to the 77 objects at a single scale and position (depicted for one object by "TRAIN") and performance was evaluated with spatially shifted or scaled versions of those objects (depicted for one object by "TEST"). The classifier never "saw" the shifted/scaled versions during training. Time interval = 100 to 300 ms after stimulus onset, bin size = 50 ms. The left-most column shows the performance for training and testing on separate repetitions of the objects at the same standard position and scale (as in Fig. 1). The second bar shows the performance after training on the standard position and scale (scale = 3.4°, center of gaze) and testing on the shifted and scaled images of the 77 objects. Subsequent columns use different image scales and positions for training and testing.

representation is thus both selective and invariant in a highly nontrivial manner. That is, although neuronal population selectivity for objects could be obtained from areas like V1, this selectivity would not generalize over changes in, e.g., position (Supporting Online Material).

We studied the temporal resolution of the code by examining how classification performance depended on the spike count bin size in the interval from 100 to 300 ms after stimulus onset (Supporting Online Material). We observed that bin sizes ranging from 12.5 through 50 ms yielded better performance than larger bin sizes (Fig. 3A). This does not imply that downstream neurons are simply integrating over 50-ms intervals or that no useful object information is contained in smaller time

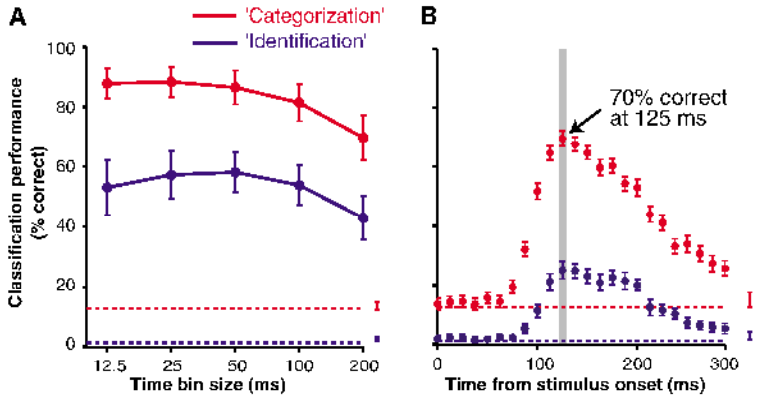

Fig. 3. Latency and time resolution of the neural code. (**A**) Classification performance (n = 128 sites) as a function of the bin size (12.5 to 200 ms, i.e., temporal resolution) to count spikes within the 100- to 300-ms window after stimulus onset for categorization (red) and identification (blue). The same linear classifier as in Figs. 1 and 2 was used. (**B**) Classification performance (n = 256 sites) using a single bin of 12.5 ms to train and test the classifier at different latencies from stimulus onset (x axis). The colors and conventions are as in Fig. 1B.

intervals. Indeed, we could decode object category at 70 ± 3% accuracy using only the spikes contained in one single bin of 12.5-ms duration at 125-ms latency (Fig. 3B). Notably, this time bin typically contained zero to two spikes (0.18 ± 0.26 spikes/bin, mean ± SD). This shows that a few spikes from a small number of neurons (essentially a binary vector with either ones or zeros) are sufficient to encode "what" information in IT neurons within behaviorally relevant time scales.

What other "types" of information are carried in the IT population? Using the readout method, we compared the information available for "categorization" versus "identification" (*18*, *37*, *38*). The time course and temporal resolution did not depend strongly on the classification task (Fig. 3); the best sites for categorization overlapped the best sites for identification; the signal-to-noise ratios for categorization and identification were strongly correlated (r = 0.54, $p < 10^{-10}$); and the same randomly selected sites could be used for both tasks (*28*). The same IT neuronal population can thus be used by downstream neurons to perform tasks traditionally considered to be different (e.g., "categorization" versus "identification").

Although anterior IT cortex is generally regarded as the brain area at the top of the ventral "what" stream, the readout approach allowed us to examine the possibility that the IT population might contain useful information about object scale and position ("where"). Our observation that IT populations convey scale- and position-invariant object category and identity information (Fig. 2) might seem to suggest that object position information is lost in IT neurons. However, it is also possible to read out — at least coarsely — both object scale and position ("where" information) based on the activity of the same population, independent of identity or category, by training the classifier to learn the map between neuronal responses and scale or position, irrespective of object identity (fig. S4A). Reading out object position or scale had a similar time course to the readout of object category (fig. S4B). There was little correlation between the ability of each IT site to signal scale/position versus object category information, suggesting that IT neurons encode both types of information (fig. S4C).

Our observations characterize the available information in IT for object recognition, but they do not necessarily imply that the brain utilizes exclusively the IT neurons (*39*) or the same coding schemes and algorithms that we have used for decoding. However, a linear classifier — which we found to be very close to optimal (*34*)—could be easily implemented in the brain by summating appropriately weighted inputs to downstream neurons. Thus, targets of IT [such as prefrontal cortex (PFC)] could decode information over brief time intervals, using inputs from small neuronal populations (e.g., ~100 neurons). It is conceivable that the dynamic setting of the synaptic weights from IT to PFC may switch between different tasks in PFC, reading out information from the same neuronal population in IT cortex (*18*). In this perspective, some neurons in IT cortex would be similar to tuned units in a learning network, supporting a range of different recognition tasks including "categorization" and "identification" in PFC (*40*).

The approach described here can be used to characterize the information represented in a cortical area such as object identity in IT cortex (*2*–*6*, *8*–*11*). Classifiers can be trained on any stimulus property and then tested to systematically examine putative neural codes for that stimulus information. Our results quantitatively show how targets of IT cortex may rapidly, accurately, and robustly perform tasks of categorization, identification, and readout of scale and position based on the activity of small neuronal populations in IT cortex.

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29. The eight groups used for categorization [toys (t), foods (fd), human faces (hf), monkey faces (mf), hand/body parts (h), vehicles (v), box outlines (b), cats/dogs (cd)] were defined before the experiments. Unsupervised clustering of neuronal responses yielded similar groups (*28*). Categorization became substantially worse upon arbitrarily defining these groups as sets of random objects (*28*). The discriminability for individual sites for passive and active viewing were similar (fig. S7, Supporting Online Material).
30. We assumed independence among neurons; this assumption should be revisited upon recording simultaneously from many neurons because correlations may contain additional information. Our estimate represents a lower bound on the information represented by small neuronal populations. However, even under these conditions, we obtain a high degree of accuracy [see also (*41*)].
31. Throughout the paper we randomly selected a given number of sites for decoding. The brain could be selectively wired such that targets of IT receive stronger input from the most relevant features. A simple feature selection step before the input to the classifier to select the sites with the highest signal-to-noise ratio (Supporting Online Material) showed that high performance levels could be achieved using a much smaller number of sites (fig. S2).
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34. A linear classifier is given by $y = sign[f(x)]$, where y

is a binary label, $\mathbf{x}$ is the input vector (coding neural activity), and $f(\mathbf{x})$ is a linear function of the form $f(\mathbf{x}) = \mathbf{w} \cdot \mathbf{x} + b = \sum_i^m c_i(\mathbf{x} \cdot \mathbf{x}_i) + b$. Training means estimating the vector of coefficients $\mathbf{w}$ and the scalar b from the training set of m $(\mathbf{x}_i, y_i)$ pairs, where $\mathbf{x}_i$ is the "input" part of each example and y_i is its associated label or "output." More complex classifiers of the form $f(\mathbf{x}) = \sum_i^m c_i k(\mathbf{x}, \mathbf{x}_i)$ had very similar performance and were no better than the regularized linear classifiers for $n \gg 64$ sites. The estimated coefficients depend on regularization and are different for different regularization techniques (*21*).

35. Multiple sources of noise can affect the encoding of information. The performance of the classifier was very robust to deletions of substantial numbers of neurons during testing, simulating neuronal or synaptic death (fig. S1A), and also to large proportions of deleted spikes (simulating failures in spike transmission or neurotransmitter release; fig. S1B).
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42. We thank M. Kouh for the object recognition model; R. Quiroga and A. Kraskov for spike sorting; J. Mayo and J. Deutsch for technical support; CSBI for computer cluster usage; and R. Desimone, N. Kanwisher, C. Koch, and T. Serre for comments on the manuscript. This research was sponsored by grants from NIH, NSF, and especially from the Defense Advanced Research Projects Agency and Office of Naval Research. Additional support was provided by Eastman Kodak Company, Daimler Chrysler, Honda Research Institute, The Pew Charitable Trusts, Whiteman fellowship (G.K.), and the McDermott chair (T.P.).

Supporting Online Material

www.sciencemag.org/cgi/content/full/310/5749/863/DC1
SOM Text
Figs. S1 to S7
References

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Neuronal Activity Regulates Diffusion Across the Neck of Dendritic Spines

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In mammalian excitatory neurons, dendritic spines are separated from dendrites by thin necks. Diffusion across the neck limits the chemical and electrical isolation of each spine. We found that spine/dendrite diffusional coupling is heterogeneous and uncovered a class of diffusionally isolated spines. The barrier to diffusion posed by the neck and the number of diffusionally isolated spines is bidirectionally regulated by neuronal activity. Furthermore, coincident synaptic activation and postsynaptic action potentials rapidly restrict diffusion across the neck. The regulation of diffusional coupling provides a possible mechanism for determining the amplitude of postsynaptic potentials and the accumulation of plasticity-inducing molecules within the spine head.

In mammalian excitatory neurons, synaptic stimulation triggers the flow of ions across the dendritic spine membrane, as well as the production of second messengers within the spine head. Buildup of signaling molecules, such as calcium or activated CaMKII (calcium/calmodulin-dependent protein kinase II), within the spine head activates regulatory cascades that lead to the modification of the enclosed synapse (*1*–*4*). Furthermore, stimulus-induced transport of proteins across the spine neck, such as CaMKII, protein translation initiation factors, and β-catenin, plays a role in synapse regulation and plasticity (*5*, *6*). Thus, the regulation of diffusion across the spine neck offers a potentially powerful mechanism to control the efficacy and modulatory state of individual synapses.

We examined the regulation of the diffusional barrier posed by spine necks in rat hippocampal pyramidal neurons. Organotypic slice cultures were biolistically transfected with the photoactivatable green fluorophore PAGFP (*7*) and the red fluorophore dsRed. Two-photon laser scanning microscopy (2PLSM) with illumination at 910 nm readily excites dsRed without photoactivation of PAGFP, revealing dendrites and spines that fluoresce in the red spectrum (Fig. 1). Focal illumination with a second laser tuned to 720 nm triggers two-photon activation of PAGFP (*8*), and the resulting green fluorescence can be subsequently monitored with 910-nm illumination. Photoactivation of PAGFP within individual spines triggers increases in fluorescence within the head that dissipate as activated PAGFP (PAGFP*) diffuses into the dendrite. The decay of the fluorescence transient in the spine head is well fit by a single exponential, yielding a time constant of equilibration (τ_{equ}) (*9*) of PAGFP* across the spine neck (Fig. 1, A to C). Repeated measurements (at 0.1 Hz) in individual spines over ~1.5 min yielded consistent values of τ_{equ} (fig. S1) with coefficients of variation (CVs) of ~15 to 20% (Fig. 1D). Conversely, τ_{equ} varied over a broad range from spine to spine (Fig. 1E, n = 11/572 cells/spines), with the majority of values ranging from 140 to 350 ms.

In a subset of spines, fluorescence did not decay appreciably in the sampling period of 1.9 s. For these spines, the barrier to PAGFP* movement across the neck was bidirectional, so that PAGFP* within the dendrite is able to diffuse away from the site of photoactivation but does not enter the spine head (Fig. 2, A and B; similar findings in 11 of 11 comparable spine/dendrite pairs). Conversely, PAGFP* diffuses from the dendrite into the heads of spines with less restrictive spine necks (Fig. 2, C and D; similar findings in 8 of 8 comparable spine/dendrite pairs). Thus, the lack of PAGFP* movement in a subset of spines results from a severe diffusional isolation imposed by the spine neck and not from aggregation or cross-linking of PAGFP within the head. Repeated measurements of τ_{equ} in these diffusionally isolated spines over prolonged periods revealed that the diffusional barrier is reversible and that large, apparently spontaneous reductions in τ_{equ} occur (Fig. 2, E and F; similar findings in 4 of 15 diffusionally isolated spines that were monitored repeatedly for >5 min).

We hypothesized that the heterogeneity of τ_{equ} results from active regulation of diffusional coupling in response to variability in neuronal and synaptic activity. Chronic manipulations of activity trigger homeostatic changes in synaptic parameters such as the number and composition of AMPA-type glutamate receptors (AMPARs) at the synapse (*10*, *11*). Consistent with our hypothesis, 24 hours of incubation in the AMPAR antagonist NBQX shifted the distribution of τ_{equ} toward faster values (8/367 cells/spines; $P < 0.01$), whereas block of $GABA_A$ receptors ($GABA_A$Rs) with bicuculline shifted the distribution toward slower values (8/556 cells/spines; $P < 0.01$) (Fig. 3A). Similar results were obtained with measurements of dsRed diffusion by fluorescence recovery after photobleaching (fig. S2). In contrast, block of voltage-sensitive sodium channels (VSSCs) (6/438 cells/spines) or NMDA-type glutamate receptors (NMDARs) (7/449 cells/spines) by incubation in tetrodotoxin (TTX) or carboxypiperazin-4-yl-propyl-1-phosphonic acid (CPP), respectively, had no effect on the cumulative distribution of τ_{equ}

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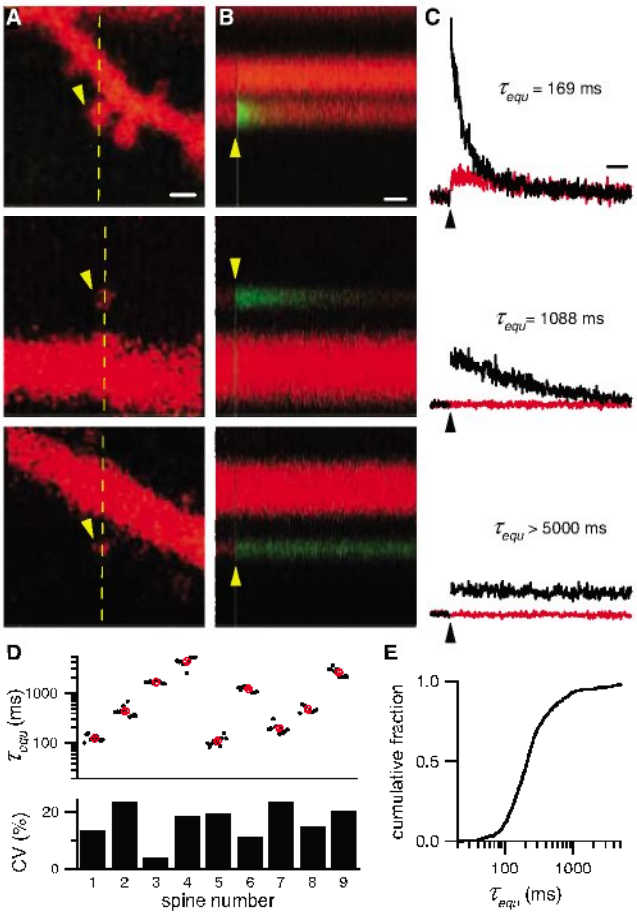

Fig. 1. Measurement of PAGFP* movement across the spine neck reveals heterogeneity of spine/dendrite diffusional coupling. (**A**) Images of spine/dendrite pairs that demonstrate strong (top), moderate (middle), and weak (bottom) diffusional coupling. In (A) to (C), the arrowhead indicates the site of photoactivation. Scale bar, 1 μm. (**B**) Fluorescence measured in line scans over the regions indicated by the dashed lines in (A) during photoactivation of PAGFP in the spine head. Scale bar, 200 ms. (**C**) Quantification of the PAGFP* fluorescence transients in the spine head (black) and dendrite (red) shown in (B). Scale bar, 200 ms. (**D**) Repeated measurements of τ_{equ} in each of nine spines (top). For each spine, the values of τ_{equ} obtained from each independent measurement (black points), the average ± SEM (red), and the CV of τ_{equ} (bottom) are shown. (**E**) Cumulative distribution of τ_{equ} for spines in control conditions.

(Fig. 3A). However, all manipulations altered the fraction of highly diffusionally isolated spines (f_{slow}), defined here as those with τ_{equ} > 2000 ms. Reducing activity levels by incubation in TTX, CPP, or NBQX decreased f_{slow} to 1.6, 1.9, and 1.8%, respectively, whereas increasing activity by block of inhibition with bicuculline increased f_{slow} to 16.4% ($P < 0.01$ for each condition compared to f_{slow} = 4.9% in control conditions, Fig. 3B). To determine whether increases in τ_{equ} are a direct consequence of blocking $GABA_AR$ signaling or are triggered by the increased action potential (AP) firing that results from the removal of inhibition, τ_{equ} was measured after incubation in the presence of both $GABA_AR$ and VSSC blockers (bicuculline and TTX). In these conditions, the distribution of τ_{equ} and the value of f_{slow} (f_{slow} = 2.0%) were the same as in the presence of TTX alone (*12*), suggesting that the loss of spontaneous $GABA_A$ currents is not sufficient to trigger modification of τ_{equ} and that secondary changes in the rate of APs or glutamatergic transmission are necessary.

τ_{equ} is determined by several factors such that $\tau_{equ} \sim VL/DA$, where V is the volume of the spine head, L is the length of the spine neck, D is the diffusion coefficient of the molecule, and A is the cross-sectional area of the spine neck (*9*). Regulation of any of these parameters might account for the observed changes in τ_{equ}. Each pharmacological manipulation had differential effects on the distributions of head widths and neck lengths (Fig. 3C and fig. S3). However, comparison of diffusional coupling across conditions for spines of similar morphology indicates that these alterations do not explain the observed

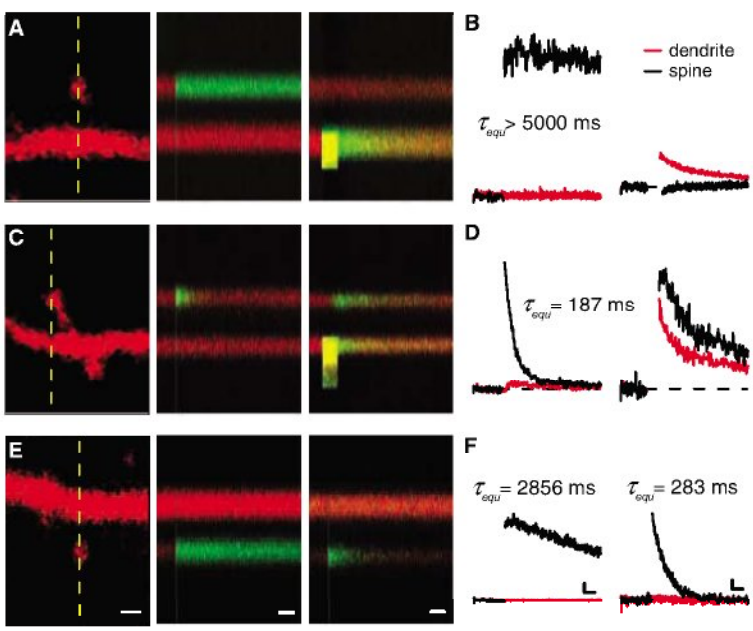

Fig. 2. The spine neck is a bidirectional and dynamic barrier to protein movement. (**A**) Image of spine/dendrite pair (left) demonstrating weak diffusional coupling and fluorescence transients obtained after photoactivation in the spine head (middle) or neighboring dendrite (right). (**B**) Quantification of the spine (black) and dendrite (red) fluorescence transients from the corresponding panels in (A) (middle and right). (**C**) Image of spine/dendrite pair (left) demonstrating strong diffusional coupling and fluorescence transients obtained after photoactivation in the spine head (middle) or neighboring dendrite (right). (**D**) Quantification of the spine (black) and dendrite (red) fluorescence transients from the corresponding panels in (C) (middle and right). (**E**) Image of spine/dendrite pair that switches from weak to strong diffusional coupling (left). Diffusional coupling was initially weak (middle) but spontaneously switched to strong (right) several minutes later. (**F**) Quantification of the spine (black) and dendrite (red) fluorescence transients from the corresponding panels in (E) (middle and right). Scale bars, 1 μm (left) and 200 ms (right and middle) for (A), (C), and (E); 10% ΔG/R and 200 ms for (B), (D), and (F).

changes in τ_{equ}. After $GABA_AR$ blockade, τ_{equ} was significantly larger than for control spines of matched neck length or apparent head width (Fig. 3D). Conversely, after AMPAR blockade, spines tended toward faster τ_{equ} than control spines with comparable morphology. Furthermore, spine neck lengths were reduced equally after NMDAR or AMPAR blockade (fig. S3), but only in the latter condition was the distribution of τ_{equ} shifted to faster values.

To determine whether cell-wide changes in cytoplasmic viscosity account for the changes in τ_{equ}, the diffusion coefficient of PAGFP* (D_{PAGFP*}) was measured in aspiny regions of thin (~1 to 2 μm in diameter) dendrites. D_{PAGFP*} (37 ± 10 μm²/s in control conditions) was consistent with previous measurements of green fluorescent protein (GFP) motility (*13*) and was constant across pharmacological conditions (fig. S4), indicating that the movement of proteins across the neck is specifically regulated in response to the manipulations of activity. Thus, changes in V, L, or D_{PAGFP*} do not account for the effects of activity on spine/dendrite diffusional coupling, suggesting that the cross-sectional area of the neck is the regulated parameter. This regulation may result from active constriction of the spine neck. Alternatively, the accessible cross-sectional area of the neck may change because of rearrangement of the cytoskeleton or the movement of organelles into the neck (*14*–*17*).

Is diffusional equilibration across the spine neck also regulated acutely by the activity of the synapse enclosed in the spine head? The effects of back-propagating action potentials (bAPs), synaptic activity, and the pairing of bAPs with synaptic activity on the spine neck diffusional resistance (Fig. 4) were measured. For these experiments, spine/dendrite diffusional coupling was measured by photoactivation of NPE-HPTS, a caged version of the green-fluorescing, pyranine-based fluorophore HPTS (*18*). Whole-cell–current clamp recordings were obtained from hippocampal pyramidal neurons that were filled through the patch pipette with NPE-HPTS and Alexa Fluor-594 and bathed in 5 mM MNI-glutamate, a caged version of glutamate (*19*). Illumination at 720 nm for 0.5 ms was used to photoactivate NPE-HPTS and uncage glutamate, and the laser power was set to generate fluorescence transients of ~20%, a 20% increase in green fluorescence relative to the resting red fluorescence (ΔG/R) in the spine head. Pairing of uncaging-evoked EPSPs (uEPSPs) with small bursts of bAPs (3 bAPs at 50 Hz) triggered increases in τ_{equ} that continued after the end of the pairing period (Fig. 4E) (n = 8/12 cells/spines, $P < 0.05$). In contrast, bAPs (n = 8/9) or uEPSPs (n = 6/11) alone, as well as repeated monitoring of τ_{equ} without stimulation (n = 6/11), had no effect on τ_{equ}. For all four experimental conditions (uEPSP/bAP pairing, uEPSPs alone, bAPs alone, and no stimulation), the analyzed spine experienced identical photoactivation and imaging laser exposures. Thus, the restriction of diffusion across the spine neck seen in response to the pairing of bAPs and synaptic stimulation represents a cellular response to the stimulus. Furthermore, because HPTS is a small polar molecule, its diffusion is similar to that of second messengers such as cyclic adenosine monophosphate.

The regulation of spine/dendrite diffusional equilibration may have several functional consequences. First, the susceptibility of individual synapses to plasticity induction

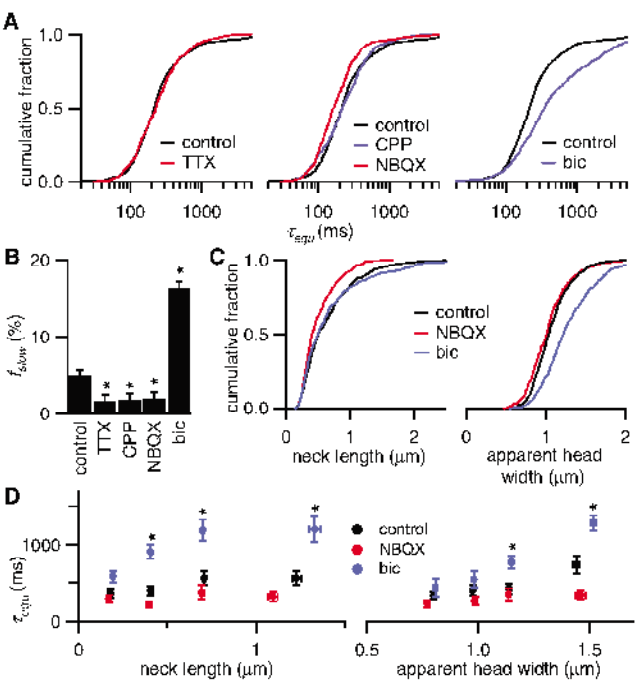

Fig. 3. The diffusional barrier posed by the spine neck is regulated by activity. **(A)** The overall distribution of τ_{equ} is unchanged when action potentials (TTX, left) or NMDAR (CPP, middle) are blocked. Blocking AMPAR (NBQX, middle) significantly decreases τ_{equ}, whereas blocking $GABA_AR$ increases τ_{equ} [bicuculline (bic), right]. **(B)** Reducing activity with TTX, CPP, or NBQX reduces the fraction of diffusionally isolated spines (f_{slow}), whereas increasing activity with bicuculline increases f_{slow}. Asterisks indicate $P < 0.01$ relative to control. **(C)** Cumulative distribution of measured neck length and apparent head width in control, NBQX-, or bicuculline-treated cultures. **(D)** Average τ_{equ} calculated for spines of similar neck lengths (left) or head widths (right) in control, NBQX-, and bicuculline-treated cultures. Asterisks indicate $P < 0.01$ relative to control.

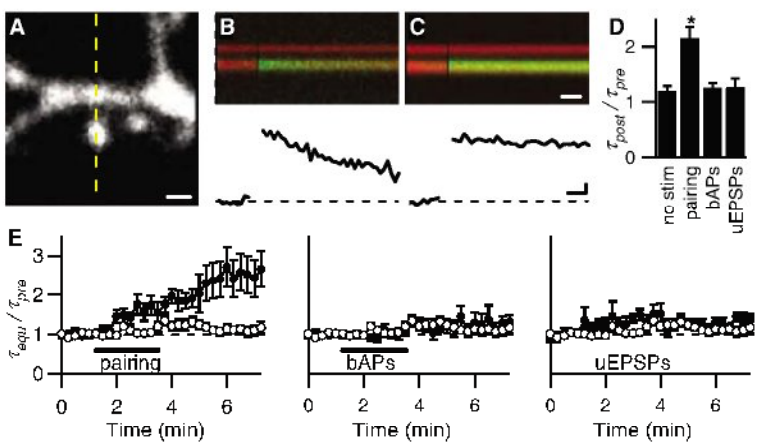

Fig. 4. Diffusion across the spine neck is restricted in response to pairing of synaptic potentials and bAPs. **(A)** Image of spiny dendrite of a neuron filled with Alexa Fluor-594 and NPE-HPTS. The dashed line indicates the orientation of the line scan used in **(B)** and **(C)**. Scale bar, 1 μm. **(B)** Average line scan fluorescence transients (top) and the quantification of the fluorescence transient in the spine head (bottom) after photoactivation in the spine head in the baseline period. **(C)** As in (B), for data collected after 10 consecutive pairings of uEPSPs and bAPs. Scale bars, 10% ΔG/R and 50 ms for (B) and (C). **(D)** Fractional change in τ_{equ} after imaging alone, pairing of uEPSPs and bAPs, or stimulation with bAPs or uEPSPs alone. **(E)** Time course of fractional changes in τ_{equ} triggered by imaging alone (open circles) or stimulation (solid circles) with paired uEPSPs and bAPs (left), bAPs alone (middle), or uEPSPs alone (right).

may be influenced by the ability of signaling molecules to move into and out of the spine head. Synaptic plasticity is typically induced by either repetitive low-frequency stimulation (*20*–*23*) or by ~1-s bursts of high-frequency stimulation (*3*, *24*, *25*) during which the spine must integrate biochemical signals. Spines with fast diffusional equilibration across the spine neck may be unable to retain second messengers or activated proteins during the interstimulus interval. Conversely, if diffusional equilibration is slow, biochemical signals generated by synaptic activation may persist in the spine head and summate during repetitive stimulation. Second messengers and many proteins involved in spine and synapse regulation are similar in size to HPTS (~500 daltons) and PAGFP (28 kD), respectively, and will experience similar diffusional barriers at the spine neck. Thus, the regulation of diffusion across the spine neck in response to changes in the activity patterns of individual cells and synapses may serve to set the threshold for plasticity induction. Second, previous estimates of diffusional coupling indicated that the barrier posed by the neck was too small to allow for a substantial voltage drop across the neck after synaptic activation of glutamate receptors (*9*). This reinforced the notion that spines function as biochemical and not electrical signaling compartments (*26*). However, the diffusionally isolated spines uncovered here have τ_{equ} approximately 10-fold greater than the population mean, suggesting a spine neck resistance approaching 1 gigohm (*9*). The stimulation of synapses housed in spines with such restrictive necks may result in depolarizations and regenerative electrical signals that are confined to the spine head (*27*).

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Tissue-Specific TAFs Counteract Polycomb to Turn on Terminal Differentiation

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Polycomb transcriptional silencing machinery is implicated in the maintenance of precursor fates, but how this repression is reversed to allow cell differentiation is unknown. Here we show that testis-specific TAF (TBP-associated factor) homologs required for terminal differentiation of male germ cells may activate target gene expression in part by counteracting repression by *Polycomb*. Chromatin immunoprecipitation revealed that testis TAFs bind to target promoters, reduce Polycomb binding, and promote local accumulation of H3K4me3, a mark of Trithorax action. Testis TAFs also promoted relocalization of Polycomb Repression Complex 1 components to the nucleolus in spermatocytes, implicating subnuclear architecture in the regulation of terminal differentiation.

Male germ cells differentiate from adult stem cell precursors, first proliferating as spermatogonia, then converting to spermatocytes, which initiate a dramatic, cell type specific transcription program. In *Drosophila*, five testis-specific TAF homologs (tTAFs) encoded by the *can*, *sa*, *mia*, *nht*, and *rye* genes are required for meiotic cell cycle progression (*1*, *2*) and normal levels of expression in spermatocytes of target genes involved in postmeiotic spermatid differentiation (*3*). Requirement for the tTAFs is gene selective: Many genes are transcribed normally in *tTAF* mutant spermatocytes. Tissue-specific TAFs have also been implicated in gametogenesis and differentiation of specific cell types in mammals (*4*, *5*). In addition to action with TBP (TATA box–binding protein) in TFIID, certain TAFs associate with HAT (histone acetyltransferase) or Polycomb group (PcG) transcriptional regulatory complexes (*6*, *7*). To elucidate how tissue-specific TAFs can regulate gene-selective transcription programs during development, we investigated the mechanism of action of the *Drosophila* tTAFs in vivo.

The tTAF proteins were concentrated in a particular subcompartment of the nucleolus in primary spermatocytes (Fig. 1). Expression of a functional green fluorescence protein (GFP) tagged genomic *sa* rescuing transgene revealed that expression of Sa-GFP turned on specifically in male germ cells soon after initiation of spermatocyte differentiation and persisted throughout the remainder of the primary spermatocyte stage, disappearing as cells entered the first meiotic division (Fig. 1A). Some Sa-GFP was detected associated with condensing chromatin (arrowheads in Fig. 1, D and E). However, most Sa-GFP localized to the nucleolus (Fig. 1, C to E), in a pattern complementary with Fibrillarin, which

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marks a fibrillar nucleolar subcompartment (Fig. 1, J and K). Staining with antibodies against endogenous Sa, Can, Nht, or Mia proteins showed similar temporal expression and nucleolar localization in primary spermatocytes, consistent with collaborative function of the tTAFs (Fig. 1, F to K) (*8*). In contrast, the generally expressed *sa* homolog TAF8 and its binding partner TAF10b were excluded from the nucleolus (*8*).

Several components of the Polycomb Repression Complex 1 (PRC1) transcriptional regulator appear in the nucleolus in spermatocytes, coincident with tTAF expression and dependent on tTAF function. Polycomb (Pc) protein expressed from a *Pc-GFP* genomic transgene localized on chromatin, but in addition became concentrated in the nucleolus in primary spermatocytes (Fig. 2, A to C) (*9*). Both *Pc-GFP* and staining of endogenous protein with antibody against Pc (anti-Pc) revealed localization to the same nucleolar subcompartment as the one containing tTAFs (Fig. 2, A to F). Recruitment of Pc to the nucleolus exactly coincided with onset of expression of the tTAFs after early G_2 phase in spermatocytes (Fig. 2, G to I; fig. S1). Relocalization of Pc depended on wild-type tTAF activity: Pc localized to chromatin but was not concentrated in the nucleolus in *tTAF* mutant spermatocytes (Fig. 2, J to L; fig. S2) (*8*). Two other components of the PRC1 core complex, Polyhomeotic (Ph) and *Drosophila* Ring protein (dRing) (*10*), also became concentrated in the nucleolus in primary spermatocytes dependent on tTAF function (fig. S2). Failure of PRC1 components to localize to the nucleolus in *tTAF* mutants was not caused by nucleolar loss because Fibrillarin staining appeared normal in the mutants (Fig. 2J). H3K27me3 laid down by action of the PRC2 complex acts as a docking site for the Pc chromodomain to recruit PRC1 and block transcription initiation (*11*, *12*). H3K27me3 localized on chromatin in spermatocytes, along with Pc. However, no H3K27me3 was detected in the nucleolus in spermatocytes (Fig. 2, M to O), suggesting that PRC1 components may be recruited to the nucleolus by a different mechanism independent of chromatin.

The tTAFs are required for activation of robust transcription of several spermatid differentiation genes, whereas the PcG proteins are known to mediate transcriptional repression. Chromatin immunoprecipitation (ChIP) suggested that the tTAFs might allow robust transcription of spermatid differentiation genes in part by counteracting repression by Pc, perhaps causing dissociation of PRC1 from cis-acting control sequences at target genes.

ChIP from wild-type testes using anti-Sa revealed enrichment of tTAF binding at three different known target genes (*fzo*, *Mst87F*, and *dj*), compared with binding at intergenic regions 10 to 20 kb away or at a tTAF-independent gene expressed in the same cell type (*cyclin A* or *sa* itself, Fig. 3A), suggesting that the tTAFs are in occupancy at target genes. Real-time polymerase chain reaction (PCR) analysis revealed ~10-fold enrichment of Sa at a target (*mst87F*) compared with a nontarget gene (*sa*) (fig. S3).

ChIP analysis also revealed that Pc protein bound to tTAF-dependent target genes in *tTAF* mutant testes, and that wild-type function of the tTAFs reduced Pc binding (Fig. 3, B and C). ChIP with anti-Pc from *can* mutant testes preferentially precipitated the three *tTAF* target promoters, compared with intergenic regions or promoters from two different nontarget controls (Fig. 3B). Quantification by real-time PCR showed more than 50-fold enrichment of Pc at the target gene *mst87F* compared with the tTAF-independent control *sa* (fig. S3). In contrast, relative occupancy of Pc at the tTAF targets was not significantly different from that at the nontargets in wild-type testes (Fig. 3C, fig. S3).

The tTAFs may act near the promoter of target genes (*fzo*, Fig. 3D) to allow expression by directly or indirectly reducing nearby binding of PRC1. ChIP using primer pairs across the promoter region of *fzo* revealed that the tTAF enriched most strongly for sequences just upstream of the transcription start site. In contrast, Pc-containing protein complexes (in *tTAF* mutant testes) enriched for a broader distribution, including sequences near and downstream of the transcription start site, consistent with localization of Pc at *Ultrabithorax* (*Ubx*) locus in wing discs and on the *hsp26* promoter in vivo (*12*, *13*).

Binding of the tTAFs at target promoters may allow expression through recruitment or activation of the Trithorax group (TrxG) transcriptional activation complex, which often acts in opposition to repression by PcG proteins (*14*). Trx, like its mammalian homolog MLL, creates an H3K4me3 epigenetic mark (*15*). ChIP from wild-type testes revealed H3K4me3 at or near the promoter regions of the three tTAF targets tested, as well as at nontargets (Fig. 3E). Analysis using primer pairs across the tTAF target *fzo* region revealed that H3K4me3 associated most strongly with sequences spanning the promoter (Fig. 3D). In contrast, ChIP with anti-H3K4me3 from *can* mutant testes did not enrich for the tTAF target promoters (Fig. 3, D and E). Quantitative PCR revealed 36-fold enrichment of the promoter region of the tTAF-dependent *mst87F* gene by ChIP for H3K4me3 in wild-type compared with *can* mutant testes (fig. S3).

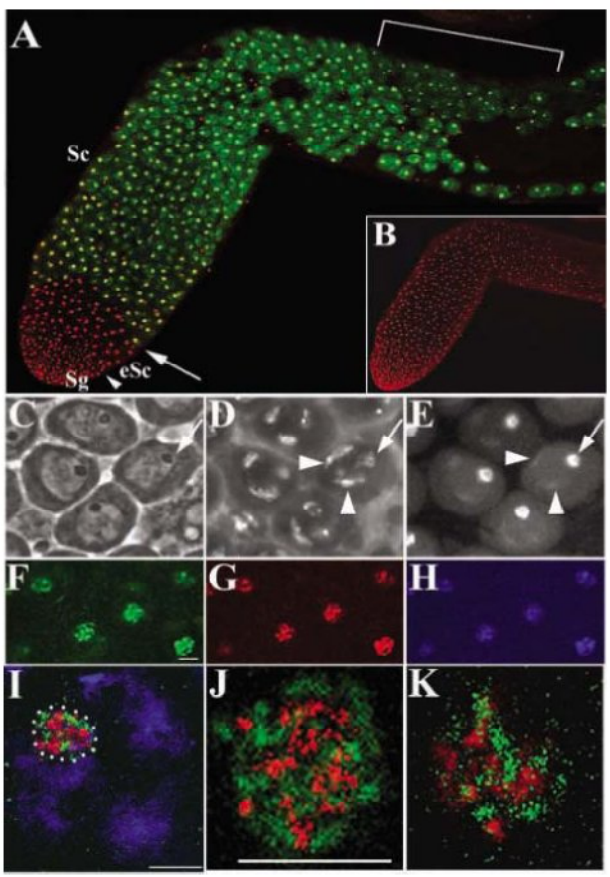

Fig. 1. Testis TAFs are expressed only in spermatocytes and concentrate in a subcompartment of the nucleolus. (**A** and **B**) Apical region of wild-type testis: (green) Sa-GFP; (red) anti-Fibrillarin, nucleolar marker in all cells. (Sg) spermatogonia, (eSc) early spermatocytes, (Sc) spermatocytes. (Arrowhead) Onset of Sc differentiation; (arrow) onset of Sa-GFP expression; (bracket) cells entering division for meiosis I. (**C** to **E**) Live spermatocytes from *sa-GFP* testis squash (C) phase contrast; (D) DNA stained with Hoechst; (E) Sa-GFP. (Arrowheads) Partially condensed autosomes; (arrow) nucleolus. (**F** to **H**) Identical field of fixed spermatocytes stained with (F) anti-Sa, (G) anti-Mia, and (H) anti-Myc (detecting expression of a *can-6myc* genomic rescue transgene). (**I**) Single spermatocyte nucleus immunostained with (green) anti-Can, (red) anti-Fibrillarin, and (blue) DAPI (4′,6-diamidino-2-phenylindole). Dotted outline: nucleolus. (**J** and **K**) Enlarged spermatocyte nucleoli: (red) anti-Fibrillarin; (green) (J) anti-Can, (K) Sa-GFP. Bar: 4 μm.

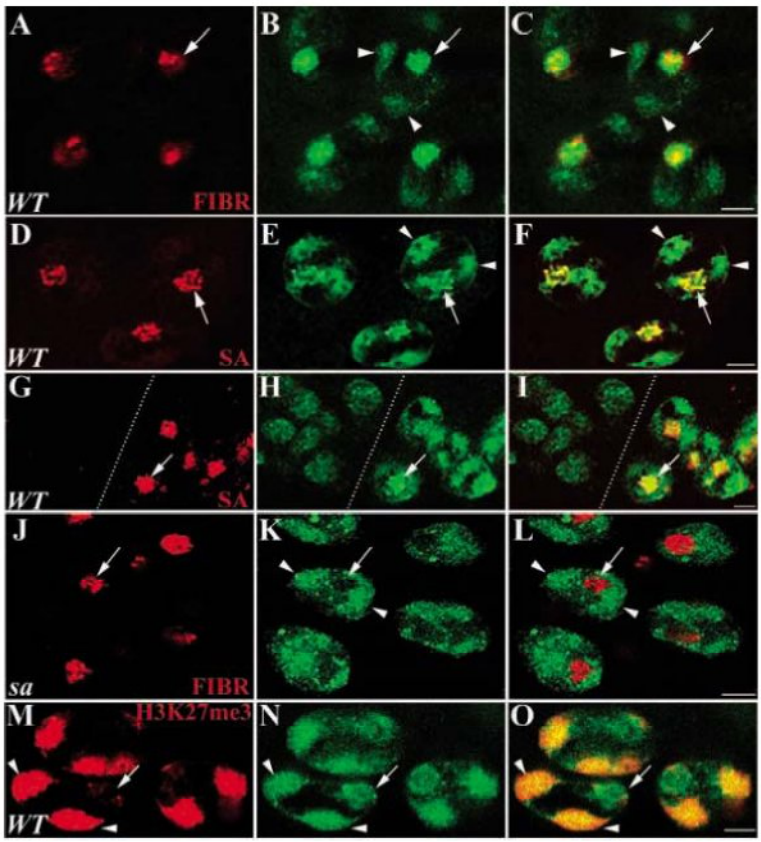

Fig. 2. Recruitment of Pc to the nucleolus in primary spermatocytes requires tTAFs. Spermatocyte nuclei showing localization of Pc-GFP (green). (Arrow) Nucleolus; (arrowheads) partially condensed autosomes. (A to F) Wild-type: (A) anti-Fibrillarin; (C) merge: complementary staining; (D) anti-Sa; (F) merge: overlapping staining. (G to I) Wild-type spermatocytes at the transition to tTAF expression; spermatocytes in early G_2 are to the left of the dotted line: (G) anti-Sa; (I) merge. (J to L) *sa* mutant spermatocytes: (J) anti-Fibrillarin; (L) merge: Fibrillarin present, but Pc-GFP absent from the nucleolus. (M to O) Wild-type: (M) anti-H3K27me3; (N) Pc-GFP; (O) merge: H3K27me3 on chromatin, but not in the nucleolus. Bar: 4 μm.

Consistent with the presence of H3K4me3 at target promoters in wild-type testes, *trx* function appeared to be required for continued expression of two different kinds of tTAF-dependent targets. Boule triggers the G_2/M transition in meiosis I by allowing translation of *twine* (*16*) and requires tTAFs for protein accumulation (Fig. 4A), setting up a cross-regulatory mechanism so that meiotic cell cycle progression awaits expression of terminal differentiation genes (*3*, *17*). When temperature-sensitive trx^1 flies grown at permissive temperature were shifted to nonpermissive temperature as adults, the Boule protein level in mutant testes substantially decreased over time at nonpermissive temperature compared with the level in wild-type flies shifted in parallel or trx^1 flies held at permissive temperature (Fig. 4, B and C) (*8*). Likewise, analysis of mRNA levels by semi-quantitative PCR revealed a ~40% decrease in transcript level for the tTAF target gene *fzo*, but not for the tTAF-independent gene *cyclin A*, in testes from trx^1 mutant flies shifted to nonpermissive temperature compared with the level in testes from similarly treated wild-type flies (Fig. 4, D and E).

In summary, occupancy of tTAFs and Pc at target promoters appeared to be mutually exclusive in wild-type and *tTAF* mutant spermatocytes, suggesting that the tTAFs may turn on target gene expression by counteracting repression by Polycomb, either directly or indirectly reducing Pc binding and allowing local action of Trx (fig. S4). Loss of function of *Pc* in marked clones of homozygous mutant cells did not restore terminal differentiation in a *tTAF* mutant background (*8*), suggesting that in addition to counteracting repression by Pc, tTAFs may also be required at the promoter region independent of Pc, possibly to recruit

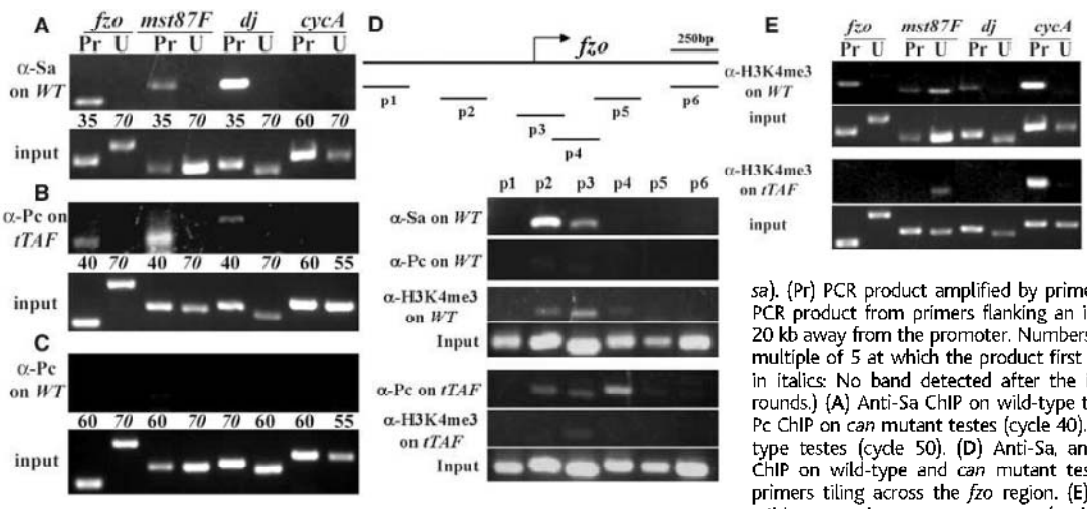

Fig. 3. Testis TAFs reduce binding of Pc to tTAF-dependent target genes. Immunoprecipitates from ChIP using anti-Sa, anti-Pc, or anti-H3K4me3 were tested for enrichment of three tTAF target genes (*fzo*, *mst87F*, and *dj*) and two non-target genes (*cycA* and *sa*). (Pr) PCR product amplified by primers flanking promoter. (U) PCR product from primers flanking an intergenic sequence 10 to 20 kb away from the promoter. Numbers below indicate PCR cycle multiple of 5 at which the product first became visible. (Numbers in italics: No band detected after the indicated number of PCR rounds.) (A) Anti-Sa ChIP on wild-type testes (cycle 35). (B) Anti-Pc ChIP on *can* mutant testes (cycle 40). (C) Anti-Pc ChIP on wild-type testes (cycle 50). (D) Anti-Sa, anti-Pc, and anti-H3K4me3 ChIP on wild-type and *can* mutant testes (cycle 35), with PCR primers tiling across the *fzo* region. (E) Anti-H3K4me3 ChIP on wild-type and *can* mutant testes (cycle 35). Products from the U-region of *mst87F* and Pr-region of *cycA* in wild-type remained in *can*, independent of tTAF activity, suggesting recruitment by an alternative mechanism. In all cases, no obvious band was visible at the same PCR cycle in mock immunoprecipitation experiments performed in parallel.

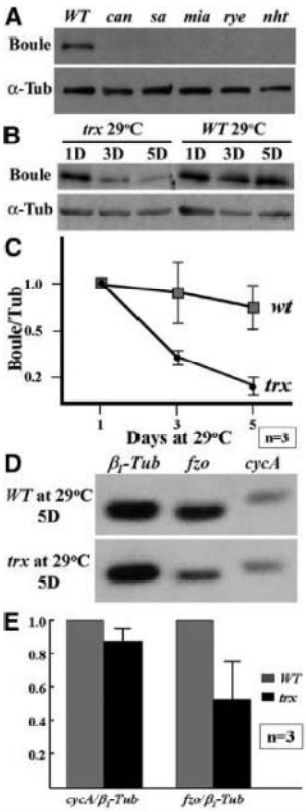

Fig. 4. Expression of tTAF-dependent target genes requires *trx* function. (**A**) Accumulation of Boule protein depends on tTAF function. Western blot of wild-type and *tTAF* mutant testes probed with anti-Boule. Loading control in (A) and (B): α-Tubulin (α-Tub). (**B**) Western blot of Boule levels over time after shift to 29°C in trx^1 or wild-type (WT) testes. (**C**) Quantitation of (B) from three independent experiments. Boule/α-Tub were normalized to 1.0 for Day 1. (**D**) Semi-quantitative reverse transcription (RT)-PCR for transcript level of the tTAF target gene *fzo* and the nontarget gene *cycA* in wild-type and trx^1 testes 5 days after temperature shift. Loading control: β_1-*Tub* (cycle 25, within the linear range for input mRNA). (**E**) Quantitation of (D) from three independent experiments; fzo/β_1-*Tub* and $cycA/\beta_1$-*Tub* were normalized to 1.0 for wild-type testes.

Trx or other cofactors for transcription activation. Transcriptional derepression by sequestration of PcG proteins has been observed during HIV-1 infection, when the viral Nef protein recruits the PRC2 component Eed to the plasma membrane (*18*). Likewise, the tTAFs may sequester Pc to the nucleolus. The tTAFs Nht, Can, and Mia are homologs of the generally expressed TAF4, TAF5, and TAF6, which were previously found as stoichiometric components of the PRC1 complex purified from fly embryos (*7*), raising the possibility that the tTAFs might associate with a population of Pc-, Ph-, and dRing-containing complexes in the nucleolus. If so, interactions in the nucleolus are likely to differ from interactions at the promoters of target genes, because the ChIP results indicate immunoprecipitation of tTAFs without Pc (Fig. 3).

The PcG and TrxG proteins act to maintain cell fates set during embryogenesis throughout development (*19*). Emerging evidence indicates that PcG and TrxG complexes also play critical roles in decisions between proliferating precursor cell fates and terminal differentiation, for example, in the blood cell lineages. In particular, the mammalian PcG protein Bmi-1 promotes proliferation and blocks differentiation of normal and leukemic stem cells (*20*), and is required for establishment or maintenance of adult hematopoietic stem cells in mouse (*21*). Transcriptional silencing by PcG action may allow self-renewal and continued proliferation of precursor cells by blocking expression of terminal differentiation genes. This repression must be reversed to allow production of terminally differentiated cells, whereas failure may allow overproliferation of precursors and eventually cancer. Although central for both normal development and understanding the genesis of cancer, little is known about the mechanisms that reverse such epigenetic silencing to allow expression of the terminal differentiation program. Our findings in the male germ line provide an example of how cell type and stage specific transcriptional regulatory machinery, turned on as part of the developmental program, might allow onset of terminal differentiation by counteracting repression by the PcG and highlight the importance of subnuclear localization in regulation of transcriptional regulation.

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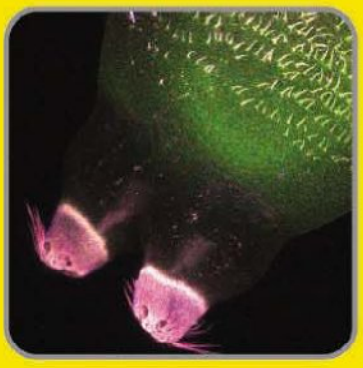

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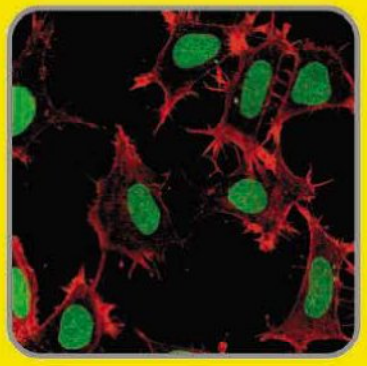

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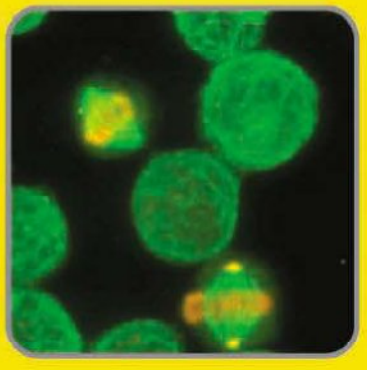

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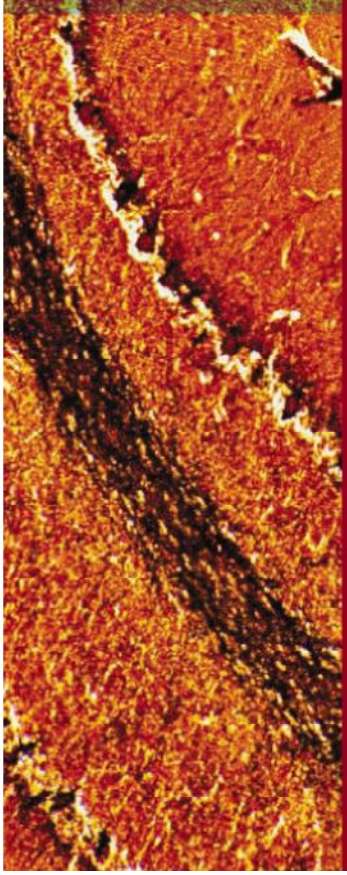
The Challenge of Neurological Disease

A variety of new tools and technologies promises to take neuroscientists closer to their goal of understanding the molecular basis of neurological diseases such as Alzheimer's and Parkinson's. BY PETER GWYNNE AND GARY HEEBNER



Neurodegenerative diseases pose some of modern medicine's most difficult challenges. Not only does our aging population face epidemic proportions of those ailments. The diseases also invade the most inaccessible reaches of the brain, making it extremely difficult to track the illnesses and determine the mechanisms behind them – the essential starting points for designing direct treatments or cures. Compounding that difficulty is the fact that the human nervous system consists of more than one trillion nerve cells.

The path to cures is plainly a long one. Nevertheless, researchers have given themselves a head start by applying such tools as agonists and antagonists, antibodies and other cell labeling reagents, microscopy, and imaging analysis systems to gain insights into the ways in which the nervous system functions – and malfunctions. "The road ahead is really exciting," declares Weiping Jiang, assistant director of **R&D Systems**. Understanding neurological diseases "is clearly the Holy Grail within science at the moment," adds Keith Watling, director of cell signaling and neuroscience for **Sigma-Aldrich**.

Two Prominent Diseases

Among the large number of neurological disorders, two have emerged as the most prominent in terms of both public knowledge and research focus. "Alzheimer's disease is the fourth largest cause of death among adults," says Chandra Mohan, senior director of technical service and senior technical writer at **EMD Biosciences** (an affiliate of German company Merck KGaA). "The major cause is believed to be deposits of amyloid peptides in the brain, which leads to the loss of neuronal cell function and the axonal flow." While Alzheimer's is a disease of aging, Mohan continues, "Parkinson's disease can happen at any age, mainly due to the loss of neurons that produce dopamine."

Current treatments of the two diseases represent hardly more than holding actions. To develop more reliable remedies, life scientists must gain a deeper understanding of their basic causes. "The two main challenges are understanding how the brain is structurally and anatomically differentiated on a cellular level," explains Bob Fasulka, director of applied optical microscopy at **Leica Microsystems**. That presents problems, though. "What scares me most about therapeutic development in neurobiology is the want of relevant in vitro models," says John Dunne, associate scientific director at **BD Biosciences – Immunocytometry Systems**. Stephanie Nickles, senior product manager at **Cambrex Bio Science**, points to another difficulty. "The availability of human neuronal cell types is very limited," she says. "Rodents are models, but they are not the real thing."

Nevertheless, the research community has begun to probe more deeply into normal and diseased brains, helped by emerging tools MORE ›››

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» advances in: Neuroscience

and technologies. "We help develop tools to help scientists assay how genes function in a cell," says Rick Ayer, product development manager at **Sutter Instruments**. "People are looking for genetic markers to identify some of the genes involved; it hasn't been a very clear picture so far," echoes Monika Trzcinska, neuroscience business development tech transfer manager at Sigma-Aldrich. "There is a need for media, culturing surfaces, and better definition of the materials in neuroscience," says Phil Vanek, BD Bioscience's director of global marketing for bioImaging. "We work with people who have a need for unique cultures and surfaces. We also have a confocal microscopy technology that's being used in the neurosciences."

Cultures, Cells, and Extracts

Every study of living cells starts with a choice of cell culture media and reagents. To ensure that cells stay alive and well during in vitro experiments, researchers and manufacturers have developed several types of growth media, some of which contain undefined biological components while others are completely defined. To study the response of nerve and other cells to changing environments, scientists can supplement these "defined" and "serum-free" media with growth factors. Companies such as **ATCC**, Cambrex, and **Invitrogen** offer those products for researchers.

Several companies also supply cells and tissue for use in the lab. **Asterand**, for example, provides human tissue samples for neurological and cancer research, while Cambrex offers a wide variety of products. "We have an extensive offering of human and animal primary cells and media kits for growth and differentiation," Nickles says. "For neural cells, we offer primary human astrocyte and neural progenitor cell systems. We also offer rat and mouse neurons and astrocytes from various regions of the brain. We guarantee our cell systems with recommended protocols for use, so the risk of failure for the customer is eliminated."

Most traditional assays require a purified cell extract. This can take several hours to prepare. It also demands great care to avoid altering the intracellular contents of living cells through mechanical forces or enzymatic degradation of proteins and nucleic acids via native DNase, RNase and protease molecules. Several vendors have responded to those difficulties by creating systems that allow scientists to use cell based assays to examine intact living cells. BD Biosciences, **Guava Technologies**, and **PerkinElmer Life and Analytical Sciences**, among other firms, have designed systems that can process large numbers of living cells under relatively natural conditions to examine molecular interactions within the cells. These systems expose cells to a compound of interest to check for any interaction with the living cells. They often use fluorescent tags to allow scientists to detect the interactions.

From Reagents to Antibodies

For several years, companies such as **Alexis Biochemicals, Biomol,** and **Tocris Cookson** have produced reagents for cell signaling and neuroscience. Other vendors, including Invitrogen, R&D Systems, and Sigma-Aldrich, offer a wide range of kits and reagents for biochemical assays and neuroscience research. "We see a lot of interest in the secretases, enzymes responsible for carving up beta amyloid," Watling says. "We have certainly got some of the inhibitors of those enzymes. We also have a beta amyloid cleaving enzyme for chopping up amyloid precursors; our kit allows customers to measure the amount of precursors."

A Resource on Aging

SAGE KE, otherwise known as the Science of Aging Knowledge Environment, offers one-stop shopping for investigators interested in the science of aging. This site features scientist-written reviews, perspectives, and case studies on neurodegenerative diseases. It delivers new stories on the latest discoveries and orientation articles on hot topics in the field. And it provides information on meetings and a variety of other sections of value to investigators.

» http://sageke.sciencemag.org

Neuroscience research also targets several of the drugs currently used to treat neurological disorders. Indeed, some of the drug candidates that fail to survive clinical trials turn out to be valuable reagents in basic research, allowing scientists to alter cellular functions in very specific ways and to target particular receptors or biomolecules. Providers of these pharmacologicals include Alexis Biochemicals, BD Biosciences, and EMD Biosciences. "We have introduced a variety of secreatase inhibitors and compounds to reduce the phosophorylation of proteins in animal studies," EMD's Mohan says. "And for research on Parkinson's disease we have various factors relevant to stem cell research."

Antibodies also play key roles in the neuroscience research lab. Tagged with labels such as fluorescein, which allow scientists to visualize them, antibodies help to identify and locate specific proteins in or on a cell. They also find use in histochemical applications, in which a cell is fixed in paraffin and sections of it stained with antibodies against a specific molecule. Researchers can identify the tagged cells using microscopy, fluorescent readers, or flow cytometers.

Companies that provide antibodies tagged with markers to eliminate the need for conjugating the antibody with a label include **Chemicon**, R&D Systems, and **Upstate**. "We have different types of antibodies – monoclonal and labeled," says R&D Systems' Jiang. "We have some that can be used in flow cytometry to follow cells and others for histochemistry under the microscope. We also have an antibody for ELISA [enzyme-linked immunosorbent assay] kits to detect how many nanograms or picograms of a compound exist."

Micromanipulation and Microscopy

Monitoring events in brain cells presents challenges more complex than those in other segments of cell biology. Sutter Instrument's Ayer gives an example. "The ability to make electrical recordings from cells in mammalian brains requires micromanipulators able to move carefully along several microns and put a pipette into or on the cell to MORE ›››

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make the recording," he explains. Other vendors of such manipulators include **Applied Scientific Instrumentation** and **Narishige**.

In addition, Sutter offers a manipulator controlled by a joystick that gives users the ability to move pipettes in three dimensions with a single control. "It has the same feel and motion as the classic manipulators, but works in an electric stepper format," Ayer explains. "It seems like a product that will gather a fairly significant part of the microinjection manipulation market over the next year or two."

Sutter also focuses on products for visual monitoring of gene function. "We have supported microscopy in a number of ways – via a powerful xenon lamp, for example, and by developing filter wheels for fluorescence microscopes," Ayer says.

Microscopy of all varieties plays a critical part in research on brain cells. "Confocal microscopy has made large advances, with its implications of high-accuracy imaging," Leica's Fasulka points out. "Scientists use fixed stage microscopy primarily to understand the very weak electrical signals and signaling pathways taking place in neural tissue. And people are using upright and inverted microscopes for multidimensional microscopy, which takes place over time or via many different wavelength probes."

Leica offers microscopes of all types, in user-friendly forms. "A novice microscope user can sit down and concentrate on the science," Fasulka says. A recent Leica advance in laser microscopy permits scientists to capture dissected materials directly into the centrifuge tube. In addition to Leica, companies such as **Molecular Machines & Industries** offer laser microdissection systems.

Other major producers of microscopes include **Carl Zeiss**, **Nikon Instruments**, and **Olympus**. Like Leica, they have not only refined the microscope but also developed digital camera systems and analytical software for data analysis.

Scientists who use microscopes to study cells face one inherent problem: Cells are colorless and translucent, and hence almost invisible under the lens of a standard light microscope. To counter the problem, companies such as **Fisher Scientific**, Sigma-Aldrich, and **Wako Chemicals** offer biological stains and dyes that allow researchers to visualize cellular structures based on their chemical characteristics. "We've introduced probes for visualizing the beta amyloids," Sigma's Watling says.

Going With the Flow

In addition to seeing cells, neuroscientists want to characterize them according to their inherent properties. For that, they use flow cytometers. Over the past three decades, BD Biosciences – Immunocytometry Systems and **Beckman Coulter** have introduced leading edge instruments.

BD aims its recent products, such as the BD FACSAria cell sorter, at scientists new to the use of cytometry. "Lots more people are sorting than ever used to in the old days," Dunne says. Another new introduction, the FACSCanto system, combines a patented optical design for enhanced signal collection on six fluorescent and two scatter parameters, digital electronics for processing up to 10,000 events per second, and a novel sample injection tube supporting carryover of less than 0.1 percent. "It is part of a family of cytometers with unusual internal controls designed to make very high end flow technology available to routine practice," Dunne explains. "The most immediate relevance of this class of cytometer is in biomarker discovery and a clinical laboratory environment."

In another recent advance, Sigma-Aldrich has introduced fibrillogenesis inhibitors – small organic molecules that can perturb large, misfolded proteins. "This represents a new approach to studying neurodegenerative diseases," Trzcinska says.

Scientists still have plenty to learn about the central nervous system and the complex disorders that affect this intricate system of highly specialized cells. But new tools and technologies have accelerated their advance toward that understanding and its application to finding treatments for neurological disorders.

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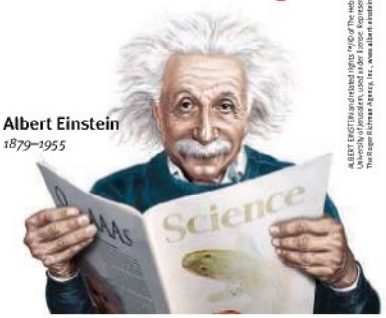

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Job Market Outlook
HOT CAREERS FOR 2006

Industry analysts and human resources specialists predict which subjects and sectors will provide the best opportunities for life scientists next year – and give advice on how best to gain employment in those areas. BY PETER GWYNNE

What prospects does the year 2006 hold for life scientists entering the job market or seeking new jobs? We asked that question of industry analysts, human resources personnel, placement officers, and other observers of the employment scene. For the most part, they give an upbeat verdict. "The general outlook is as promising as it has ever been," says Matt Gardner, president of the Bay Area Bioscience Center (BayBio).

However, Gardner and other authorities point out that the profile of life science has changed significantly in recent years. The encouraging prospects owe much to the biopharmaceutical industry's need to push promising drug candidates into clinical trials, thereby refilling declining drug pipelines. Biotechnology firms, rather than giant pharmaceutical companies, have begun to generate many new drugs, and hence provide job opportunities for life scientists. On the other hand, support for research by the National Institutes of Health has leveled off after a period of spectacular annual increases, a phenomenon that affects both government and academic research. At the same time, countries outside the United States have begun to cultivate their own biopharma industries, many of them competing directly with American firms.

In light of those trends, life scientists seeking jobs must perform the seemingly self-contradictory tasks of thinking in interdisciplinary ways while gaining narrowly specialized competences. "Companies need scientists with a broad background in several disciplines, but they have a broad need for scientists with special skills as well," says Chris Jock, vice president and general manager of scientific staffing firm Kelly Scientific Resources. Bill Lindstaedt, director of the Career Center at the University of California, San Francisco (UCSF), explains what that means to would-be employees. "Clinical researchers, toxicologists, and pharmaceutical chemists seem to be pretty popular right now," he says. "The basic molecular biologist seeking jobs on the discovery side might be facing more competition." In short, this isn't your old professor's world of life science. CONTINUED »

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ELEANOR BABCO

Academic Shortfalls

An issue associated with academic training will have a clear impact on the prospects for life scientists' employment in the coming year. "Universities are not kicking out the numbers of people necessary to support the life science industry," Jock says. "Industry is trying to get universities and colleges to craft programs to meet their particular needs." Those efforts seem destined to fail, at least for a while. "Academics can't distinguish between education and workforce development," says A. Stephen Dahms, executive director of the California State University Program for Education and Research in Biotechnology (CSUPERB). "Academic faculty members do what they do best: train people for the bench. But they do not understand the skill sets that are absolutely critical for drug development."

Another factor might have a negative impact on life scientists' attitudes toward careers in their field, particularly in government and industry in the United States. Recalls of Cox-2 inhibitor drugs during the past year have raised questions about the credibility of the U.S. Food and Drug Administration's (FDA's) regulation of new drugs and suspicions that pharmaceutical companies have been overly eager to put fresh remedies on the market.

On the one hand, the recalls and related incidents could increase the availability of jobs. "The opportunity is there to get it right next time," points out John Hodgson, director of Critical I Limited, a British based firm that helps clients in commercial and academic life science to mobilize innovations. "It may impel companies to be more thorough in the early stages of their testing. That will attract young researchers." Steve Burrill, CEO of Burrill & Company, a merchant bank that concentrates on life sciences, sees another side of the coin. "On the negative side," he says, "concerns about drug safety will make the FDA more cautious and might start to dry up some of the venture capital for biopharmaceuticals."

Issue for Idealists

Michael Gottesman, deputy director for intramural research at the National Institutes of Health, points to one possible effect of the drug industry's problems on idealistic young life scientists. "There are people who want to conduct their research without having to worry about whether it will lead to a marketable product in the short term," he explains. "The problems with the pharmaceutical industry may make government research labs more attractive as places in which to do highly innovative, high-impact research." So far, however, little evidence has emerged of reduced enthusiasm for pharmaceutical careers. "I haven't seen any decline in applicants because they don't want to get involved with the industry," says Eleanor Babco, executive director of the Commission of Professionals in Science and Technology.

Projected figures warrant optimism about careers in life science in the United States. The U. S. Bureau of Labor Statistics forecasts that 252,987 individuals will work as life scientists in 2012, up from 213,994 in 2002. During that period, employment of life scientists will grow at a rate three times as fast as the average for all jobs.

Some demographic regions have continuing and spectacular success in creating new jobs. "In the Bay Area we create a company every 10 to 14 days, characterized by a high number of Ph.D.s," Gardner says. Europe's life science industry also looks ready to revive. "We have data to show some contraction in the European biotech sector in the last couple of years, largely due to underfunding and mergers and acquisitions," Hodgson says. "But the investment is increasing again, partly owing to government moves around the Lisbon agenda [a goal of spending 3 percent of gross domestic product on R&D to which member countries of the European Union agreed in 2000]. This suggests that life science jobs are on the up and up. To get to the Lisbon target by 2010 will need job increases in 2006."


STEVE BURRILL

International Factors

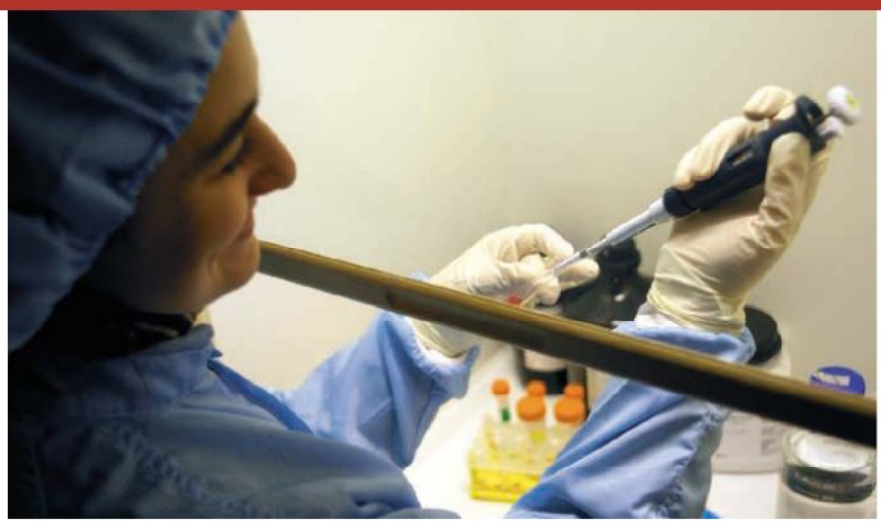
Other international factors offer less encouragement for life scientists seeking jobs in Europe and North America. "There are significant opportunities in outsourcing to China and India, particularly in respect to drug discovery, early development, and the preclinical and clinical sectors," Burrill says. "I see big growth there, but perhaps some decline in Europe and North America. It may not be economic to develop a drug today in the U.S. and Europe, where the market size is about equal to the cost of development. But it may be economic to develop the same drug in India or China."

Overseas nations have also begun to create their own biotechnology industries. "On a global scale there are more biotechs outside the United States than in it," Burrill continues. "The field is growing in Japan, Australia, New Zealand, Cuba, the Benelux countries, India, China, Malaysia, and Korea."

The internationalization of life science manifests itself in commitment to one of the hottest fields in present-day research: stem cells. Although the U.S. government puts stringent restrictions on funding for research on embryonic stem cells, individual states such as California have set up institutes to perform that work and general stem cell studies. "And around the world we'll see stimuli to get involved in stem cell work," Burrill predicts. "Lots of countries have made that a priority. The Koreans, Chinese, Singaporeans, and Russians are giving a lot of support to stem cell research."

Stem cells will remain largely the province of academic researchers next year. But industry also looks forward to change, particularly in drug discovery. "Big pharma has been fairly resilient, with pharmaceu-

Job Market Outlook
HOT CAREERS FOR 2006



tical firms continuing to hire for drug development. That's going to continue, but at a slower pace," Babco forecasts. "Most of the new drugs will come through biopharmaceuticals – drugs produced through biotechnology. That will involve not just discovery but also further development and manufacture."

Interdisciplinary Training

As that comment indicates, many of the most promising opportunities in pharmaceuticals will involve the post-discovery phases of drug creation. "Because everybody's concerned about the amount of time and money it takes to produce a drug and get it to market, there will probably be a growth of jobs involved with getting drugs into clinical trials and drug safety," Babco says. "Qualifications in regulatory affairs, validation, and quality control will be helpful."

Practically, that means that life scientists must obtain more interdisciplinary training than they have in the past. "It's not enough to have a Doctorate in cell biology or molecular biology," Babco asserts. "You'll need very specific experience in several areas. You have to have a very strong background in cell structure and cell biology but also a multidisciplinary feel."

UCSF's Lindstaedt agrees. "Having more than one field of training is important for postdocs," he says. "The Ph.D. is all about getting depth; the postdoc should add some breadth to that depth." UCSF has a strategy for achieving that. "Next year our Office of Postdoctoral Education will roll out two cross-training programs to promote interdisciplinary research," Lindstaedt says. "One will help basic scientists understand the molecular basis of disease and the other is an emerging techniques course that will give them an understanding of how to do a technique and to see what its applications are."

The same attitude pervades noncommercial institutions. "We are specifically focusing on two areas of interdisciplinary science," NIH's Gottesman says. "One is translational medicine: lab-based science with translation to human experience. We're looking for people interested in doing basic research and applying it to human problems. The other is combining biology with the physical sciences – physics, engineering, math, and computer sciences. People who have skills in those areas will have little trouble getting jobs in the future."

The term "interdisciplinary" doesn't refer only to understanding of different fields of science. "Scientists have to be prepared to work cross-functionally, with business development people, for example," Lindstaedt says. Adds Jock of Kelly Scientific. "You need a good understanding of the product development cycle, the ability to work in a multifunctional arena, and you need to be able to articulate in a business setting. This will help to guarantee funding of your project."

Hot Fields and Subfields

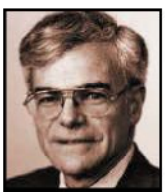
What disciplinary fields and subfields will offer the greatest potential for employment in 2006 and beyond? "Certainly at the molecular level, research on signal pathways and chemistry are important," Jock says. "Scientists need a broader understanding of the interface between chemistry and biology. You also throw in a good, healthy dose of informatics and bioinformatics, which companies use to make go–no go decisions on projects. Most biologists understand informatics, but not at the level that industry needs; that will be part of their ongoing education and training."

While the American supply of informatics specialists has increased in recent years, their transatlantic cousins can hardly cope with demand for their services. "If there's one overarching area that's a hot field in Europe, it's probably bioinformatics – particularly calculating the level of trust one can have in information," Critical I's Hodgson says. "What the field needs are people with a strong biology background overlaid with an understanding of informatics who can make sure that the natural fuzziness of biology is defined. You also need them to develop ways of making data from different sources talk to each other."

Burrill sees the development of what he calls "theranostic drugs" – remedies targeted at specific populations of patients – as a critical factor in the future employment of life scientists. That work will demand individuals trained in certain key subdisciplines. "In addition to stem cells, the pharmacogenomics and pharmacogenet- CONTINUED »

ics arena is expected to be hot," Burrill says. "Therapeutically, areas like memory and obesity are going to be significant spends. Diseases of the underdeveloped world are increasingly important. Cardiovascular diseases and cancer are still significant killers and have meaningful funding. And technologies for drug delivery will be important." Babco points to another subfield relevant to modern drug development. "We'll continue to have plenty of action in anything to do with proteomics, such as protein kinases, the control switches for many cellular functions," she says.

In Europe, meanwhile, hot job opportunities will depend partly on geographic location. "Some Danish companies have had to move to Switzerland because of a shortage of chemists in Denmark," Hodgson says. "In France, there's a lack of clinical trial specialists."


MICHAEL GOTTESMAN

Institutional Imperatives

Geography represents just one demographic influence on future job prospects. Institutional factors also play a role. In some areas, such as government and academe, opportunities will grow slowly, if at all. Other sectors, such as small biotechnology firms, anticipate marked increases in their need for life scientists.

"At NIH, we're facing a period of relatively flat budgets," Gottesman says. "But we're well aware that it's incredibly important for us to recruit new people into biology. Our intent is to continue to recruit at historical levels – 30 to 35 tenure-track positions per year." Recruitment should also benefit from attempts to counter the perceived "graying" of the staffs of individual institutes. "There's a real effort going on to identify new leadership to replace those who are retiring," Gottesman continues.

Universities seem unlikely institutions for expanded job opportunities in the near future. "I don't see the job market for academics improving greatly next year," UCSF's Lindstaedt says. "Not only do the big state universities have flat support from NIH; they also have a problem getting funds for more tenure-track positions." However, academic institutions should benefit from the burgeoning interest in research on stem cells. "We expect to see a significant increase in spend in the stem cells arena, mostly in the academic sector," Burrill says.

The biopharmaceutical industry will also increase its spending as it moves new drug candidates into development and clinical trials. "The assumption is that the industry will grow at a 10 percent annual clip in Northern California for the next few years," says BayBio's Gardner. That growth will provide job opportunities in companies of all sizes. "Very large companies say they'll never have enough research associates and enough manufacturing associates," Gardner continues. "Small companies are far more specific in their needs. They want people in product development, for example, and offer very targeted research positions."


JOHN HODGSON

Education or Work Force Development

But do graduate life scientists have the skills they need to survive and thrive in the corporate world? CSUPERB's Dahms thinks not. "Academics are contributing to the workforce, but many of the necessary skill sets come with foreign nationals," he says. "People with those skill sets have good science backgrounds but are well involved in the multiple steps that represent the environment in companies, particularly small r, large D companies."

As Dahms sees it, students can't pick up those skills in the typical two-hour course in industry that many life science departments offer. Instead, he says, "they have to bridge past the constraints set by their advisers to look at the offerings of colleges of engineering and of business, which have a good mode of thinking to guide students in the right direction."

Dahms, who a is board member of the Council of Biotechnology Centers, a section of the Biotechnology Industry Organization, recommends another way in which life science graduates can bridge the constraints. "Take high end professional Master's degrees," he suggests. "These two-year programs have courses in project management, negotiation, and other skills. In essence, the programs have 40 percent to 50 percent of the same content as business school courses." Babco agrees. "You need people who can interface among all the different specializations, including those relevant to business as well as science," she says. "Professional Master's degrees can be useful for project directors."

The programs focus narrowly on such areas as management of drug development, reimbursement affairs, and regulatory affairs. "These are areas so incredibly on target that they have zero unemployment," Dahms says. His comments on the courses stem from firsthand knowledge. "I head a Center for Biodevice Development at San Diego State University with a professional Master's degree," he explains. He adds one caution: Because professional Master's programs remain fairly rare, students must work hard to find them. "Some of them do not market themselves extensively," he says, "as they would be overwhelmed."

Certificate Programs

At a slightly lower level, undergraduate departments and community colleges offer certificate programs in specific areas of expertise. "The programs fit a demographic defined by skills and skill sets," Gardner explains. "Those programs are full of students with Bachelor's degrees. Qualified chemists and life scientists have to go back to take short sources in good lab practice, for example."

The availability of courses geared to the skills and mindset that industry needs raises the issue of how far life scientists need to take their academic training. "I think there's increased need in the industry for some of the more pedestrian skills, which may be lower even than Bachelor's degrees," Burrill says. Gardner agrees. "As some companies in this arena reach the mature organization stage, the profiles of their new hires are

changing," he says. "Genentech, for example, is reversing the stratification of its workforce, from 70 percent Master's degrees and above to 70 percent Bachelor's degrees and below."

Plainly, many of the workers in the biopharmaceutical vineyard have no need of higher qualifications. "You certainly need a lot of people who will work the scientific machines," Hodgson points out. "Not all of those will need to be as research minded as the Ph.D. or postdoc. There's a lot of scope for people coming in at an earlier level who look to combine a basic science grounding with experience in business or development work." Scientists without Ph.D.s also have opportunities for work beyond the laboratory. "We'll be able to use specialists to communicate with lawmakers, the press, and the public," Babco says. "They'll have to communicate the value of new drugs; they don't necessarily need a Ph.D. to do that."

Jock foresees an increasing stratification in the scientific workforce that might reduce the value of Master's degrees – apart from the new professional versions. "There's going to be a need for Bachelor's qualified individuals with solid science training, but you'll also need the Ph.D.s," he says. "You'll see a bimodal distribution, with Bachelor's graduates trained in specific fields such as RNA interference or signaling pathways and the Ph.D.s who can be project leaders and investigators."


CHRIS JOCK

Collegiality and Communications

Scientific qualifications represent just one aspect of job applicants' appeal. Employers in academe, government, and industry uniformly look for evidence of job seekers' communications skills and collegiality. "People-to-people skills like these are absolutely essential skill sets, within the company and for dealing with federal agencies," Dahms declares.

The ability to collaborate has become particularly critical. "You need both collegiality and communications skills, because industrial life science is a team act," Hodgson says. "Equally important is flexibility both in thinking and the ability to switch from one project area to another."

Government work provides some scope for isolated individual initiative. "Because there's a lot more team science, people must be able to work in teams. We also need leadership skills, as teams need leaders," NIH's Gottesman explains. "That doesn't rule out the brilliant lone investigator, though. There will still be opportunities for them in government labs and universities."

Industry has much less time for solo scientists. "Being able to work in large groups where your result is pretty much important to the overall mission goal is important. You have to be a link in the chain rather than carving yourself out as a separate island," Jock says. "That was less evident 10 to 15 years ago, but it has changed as the global economy has developed. You might have to interact with colleagues across the U.S. and across continents."

Job seeking scientists must recognize the reality of globalization. "If we set up a company now, we are global from the time we start," Burrill says. "We can license from anywhere in the world. Our intellectual property is a global issue. Capital is very fluid. Disease knows no borders. And the need to be Internet- and global communications–savvy is a much, much higher priority today than it was a few years ago." Native English speakers have one advantage here. "English is the language of science and the language of business," Burrill continues. "Regardless of where you are in the world, it's important to be fluent in English."


BILL LINDSTAEDT

Speaking Up

Whatever their first language, industrial scientists must be prepared to speak up for themselves and their projects. "You have to have the sensitivity necessary to maximize receptiveness to your ideas," Jock continues. "You have to be somewhat of a salesman." Hodgson echoes that point. "Industrial research is about getting to the next decision point," he says. "Any call might terminate a project or an entire R&D program. The ability to fight your own corner is important."

Lindstaedt points out several ways to do that. "Learn negotiation skills – being able to bring a group to an agreement," he advises. "Also develop your ability to delegate tasks, to select good candidates in hiring, and then to motivate them to follow up on projects."

Scientists planning to seek jobs in 2006 should also devote themselves to due diligence on potential employers. "Preparing yourself for a discussion about a company's work, an openness to its assignments, and an understanding of how a commercial company works are all important," Gardner says. "You can find enough information in public filings to be very well versed in a company's affairs – its market, its competition, and its prospects. There's no excuse for ignorance."

Applicants need also recognize how corporations deal with their resumes. "Because companies are increasing the number of electronic resumes they receive through the Internet, it's going to be important for applicants to research employers for the keywords they'll need to get past the resume software," Babco warns. "Candidates will have to do more homework to make sure that the software doesn't bounce out their resumes."

Gardner offers one final piece of advice to job seekers. "You could never do enough practicing and preparing for your interviews," he advises. The jobs will be out there next year, but you'll have to pursue them with vigor and enthusiasm.

A former science editor of Newsweek, *Peter Gwynne writes about science and technology from his base on Cape Cod, Massachusetts, U.S.A.*

UNIVERSITY OF CALGARY

The Chair in Spinal Cord Research

The **Faculty of Medicine** invites applications and nominations for an exciting full-time academic position as The Chair in Spinal Cord Research. The successful candidate will be appointed in the Department of Clinical Neurosciences and will become a full member of the Hotchkiss Brain Institute.

The successful candidate will be an established investigator with a distinguished international reputation in spinal cord research and proven leadership in education in the field. The incumbent will provide leadership in spinal cord research in Southern Alberta, establish effective education and research links with national and international research groups and centres with a view to accelerating a cure for spinal cord injury, as well as attract outstanding trainees and scholars in this field. Eligibility for licensure in the Province of Alberta and privileges within the Calgary Health Region are required if the selected individual will provide patient care.

The selected candidate will be expected to compete for salary support and establishment funding from the Alberta Heritage Foundation for Medical Research. We anticipate generous start up funds will be available to a qualified candidate.

With over 400 full time members, the Faculty of Medicine is a leader in health research with an international reputation for excellence and innovation. A new research facility opening in 2006 will allow researchers to investigate scientific questions collaboratively in a unique setting that facilitates multidisciplinary studies with state-of-the-art investigative tools. Calgary is a vibrant, multicultural city of ~1,000,000 near the Rocky Mountains, Banff National Park and Lake Louise.

The Department of Clinical Neurosciences and the Hotchkiss Brain Institute have joined forces to enhance their collective efforts in world-class research and innovation to rapidly advance patient care – in the areas of Stroke, Multiple Sclerosis, Spinal Cord and Nerve Regeneration, Epilepsy, Parkinson's Disease and Brain Imaging – through a cohesive, integrated and outcome focused approach. See our website www.hbi.ucalgary.ca for more information.

Please submit curriculum vitae, a statement of research interests and goals, and the names of three referees by **December 31, 2005,** to:

Dr. P.A. Sokol
Vice Dean, Faculty of Medicine, 3330 Hospital Drive N.W., Calgary, Alberta, Canada T2N 4N1

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada.
The University of Calgary respects, appreciates and encourages diversity.

www.ucalgary.ca

McGill University
Department of Psychology
Assistant Professor Position in Behavioral Neuroscience

The Department of Psychology of McGill University seeks applicants for a tenure-track position at the Assistant Professor level in **Behavioral Neuroscience**. Our current strengths within this broad domain are in the areas of vision, learning and memory, the psychopharmacology of reward and reinforcement, and the biological basis of pain. Applications in any area of Behavioral Neuroscience are encouraged to apply. The Department has excellent facilities for interdisciplinary research through its links with related academic departments and research units in the McGill University Health Centre including the Montreal Neurological Institute.

Consideration of applications will begin **November 15, 2005** and continue till an appointment is made. Applicants should present evidence of the ability to establish a record of significant, externally funded research productivity. All applicants are expected to have an aptitude for undergraduate and graduate teaching. Applicants should arrange for three confidential letters of recommendation to be sent to the address below. A curriculum vitae, description of current and proposed areas of research, selected reprints of published or in press research articles, a description of areas of teaching competency, interest, and approaches, and other relevant material, should also be sent to

Chair, Behavioral Neuroscience Search Committee
Department of Psychology
McGill University
1205 Dr. Penfield Avenue
Montreal, Quebec, Canada H3A 1B1

All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority. McGill University is committed to equity in employment.

UNIVERSITY OF ALABAMA AT BIRMINGHAM
DEPARMENT OF NEUROBIOLOGY
EVELYN F. McKNIGHT BRAIN INSTITUTE

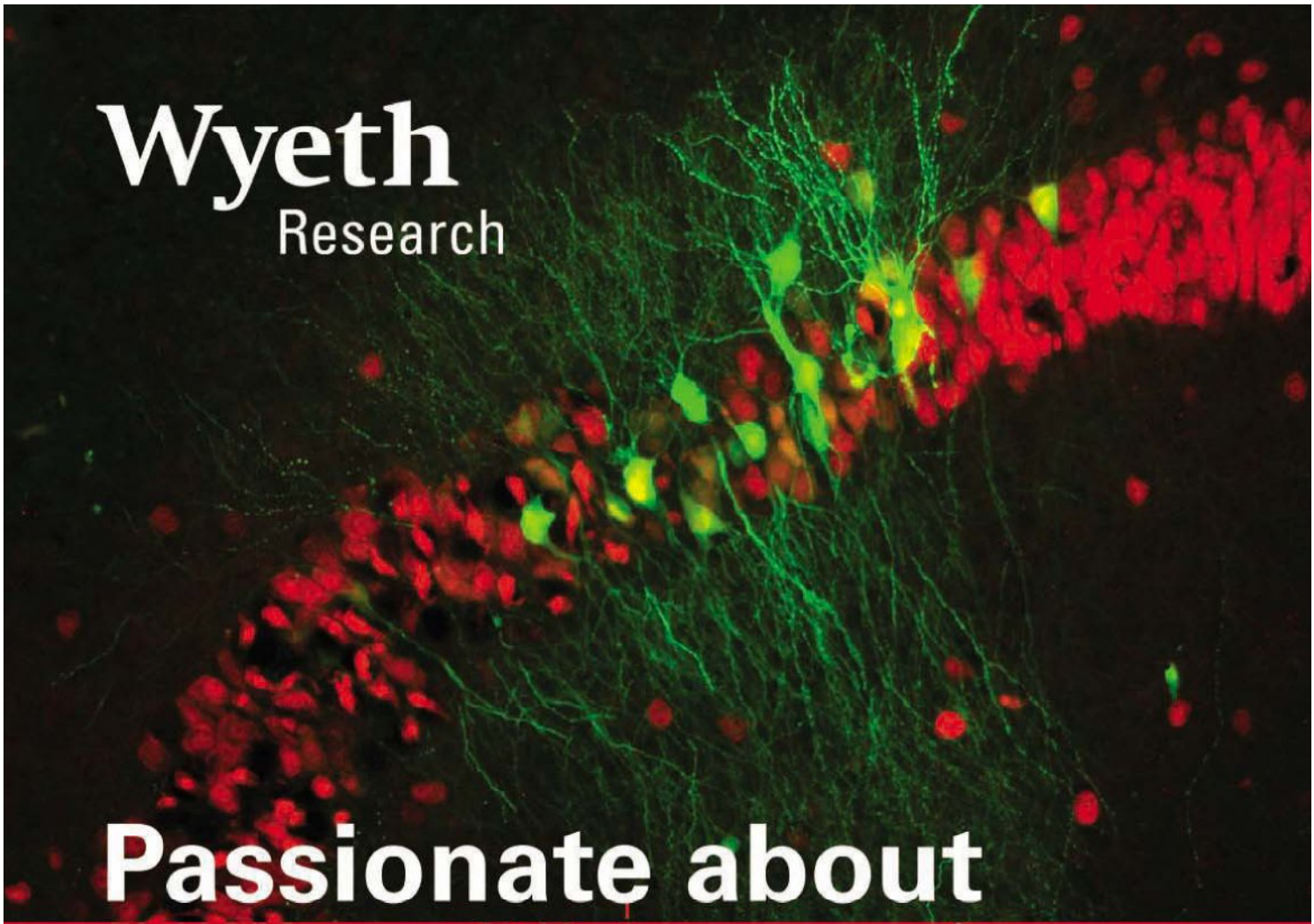
The Department of Neurobiology at UAB invites applications for tenured or tenure-track positions at the rank of Assistant, Associate, or Full Professor. The Department is under new leadership and undergoing significant expansion. We are recruiting outstanding scientists in the research areas of cell signaling, synaptic plasticity, learning and memory, and learning/memory disorders, with an emphasis on scientific excellence regardless of research area or faculty rank.

The Department of Neurobiology offers a collegial and creative environment and will be housed in the Evelyn F. McKnight Brain Institute comprising three floors (approximately 75,000 square feet) of the new Annette and Richard Shelby Research Building. UAB is one of the leading academic medical centers in the country and is currently ranked 18th in NIH funding among all US medical schools. More information can be found at **www.neurobiology.uab.edu**. Deadline for applications will be **January 31, 2006**. Senior applicants should submit a CV. Junior applicants should provide a CV, description of proposed research and names of 3-5 references to:

Dr. J. David Sweatt
Chairman, Department of Neurobiology
Director, McKnight Brain Institute
University of Alabama at Birmingham
1719 6th Ave South
CIRC 516
Birmingham, AL 35294-0021

E-mail: dsweatt@nrc.uab.edu

The University of Alabama at Birmingham is an Affirmative Action/ Equal Opportunity Employer. Women and minorities are encouraged to apply.



Wyeth
Research

Passionate about *Neuroscience*

Career Opportunities in Discovery Neuroscience
Wyeth Research, Princeton, NJ

At Wyeth Neuroscience, our vision is to be at the forefront of discovering and developing novel medicines that improve the quality of life for patients suffering from neuropsychiatric and neurodegenerative disorders.

We are passionate about neuroscience and in achieving scientific excellence. Our collaborative and rewarding culture provides the driving force behind one of the worlds most innovative research pipelines across a number of disciplines
including: Depression & Anxiety, Neurodegeneration, Neuroregeneration, Neuropharmacology & Neurophysiology, Schizophrenia & Bipolar Disorder and Pain.

If you are passionate about neuroscience, e-mail your resume to chiaret@wyeth.com
(source code, NPT) or visit our website at www.wyeth.com/careers
Equal Opportunity Employer M/F/D/V

"Leading the way to a healthier world"

NORTHWESTERN FEINBERG SCHOOL OF MEDICINE
DEPARTMENTS OF
NEUROLOGY AND RADIOLOGY
FACULTY POSITION

The Neuromuscular Disorders Program of Department of Neurology announce a new search to recruit outstanding individuals for full-time, tenure-track, appointments at the level of **ASSISTANT PROFESSOR** depending upon prior experience and research accomplishments. Applications will be considered in areas of **novel therapeutic applications to ALS and other neurodegenerative conditions and imaging of neurodegeneration.**

The Ph.D. or M.D. appointees are expected to have demonstrated exceptional potential in therapeutic or imaging research. Responsibilities of the positions are to develop dynamic, independently funded research programs and to participate in medical, graduate, and postgraduate teaching. High quality laboratory space and excellent start-up support will be provided. Salary will be negotiable depending upon experience.

The appointees will have access to new state-of-the-art animal facilities and to shared facilities for tissue culture, cell imaging, transgenic and knockout projects, monoclonal antibodies, gene and protein microarrays, structural biology, and biotechnology. The candidate for imaging will have a primary appointment in the Department of Radiology with access to all clinical and research facilities, including the Center for Advanced Imaging Research (CAMRI) containing 1.5T and 3T research MR scanners and an animal angiographic room. Plans are also underway for a 7T small animal MR research suite.

Additional information about the Neuromuscular Disorders Program can be found on our Web pages (www.neurogenetics.northwestern.edu). Applicants must include the following materials: (1) current C.V. and list of publications, (2) brief statement of research interests (three pages or less), and (3) three letters of reference sent on their behalf to:

Teepu Siddique, M.D., (t-siddique@northwestern.edu)
Chair, ALS Search Committee
Northwestern University Feinberg School of Medicine
Tarry 13-715, 303 E. Chicago Avenue
Chicago IL 60611

Completed applications must be received by **January 31, 2006**. Appointments will commence on or after June 1, 2006.

Northwestern University is an Equal Opportunity/Affirmative Action Educator and Employer and invites applications from all qualified individuals. Applications from women and minorities are especially sought. Hiring is contingent upon eligibility to work in the United States. Please refer to Academic Search number P-128-06.

PENNSTATE

FACULTY POSITION IN MOLECULAR AND CELLULAR NEUROBIOLOGY
The Pennsylvania State University

The Department of Biology and the Huck Institutes of Life Sciences at Penn State University invite applications for a faculty appointment at the Assistant to Full Professor level (open rank). We seek an outstanding scientist in Molecular and Cellular Neurobiology whose research is internationally recognized and complementary to existing areas of expertise in our Department. For further information about the Biology department, see **http://www.bio.psu.edu**.

Please submit letter of intent along with a curriculum vitae, statements of research plans and teaching philosophy, copies of relevant publications and arrange for three letters of recommendation to be sent on your behalf to: **Chair of Neurobiology Search Committee, 208 Mueller Laboratory, Box W, Penn State University, University Park, PA 16802**, email: **sjgookin@psu.edu**. Electronic applications submitted by email are preferred. Review of applications will begin **December 1, 2005**.

Penn State is committed to Affirmative Action, Equal Opportunity, and the diversity of its work force. AA/EOE.

Center for Learning and Memory
Institute for Neuroscience
The University of Texas at Austin

The Center for Learning and Memory at the University of Texas at Austin invites applications for a number of tenure track faculty positions at the Assistant, Associate, and Full Professor levels. Fields of interest include cellular, molecular, behavioral, and computational neuroscience in the general areas of plasticity, learning, and memory. Successful candidates will be expected to develop and maintain an active research program within an exciting and vibrant academic environment. Academic appointments will be made in the appropriate academic unit within the Colleges of Natural Sciences, Liberal Arts, Pharmacy, or Engineering. The positions carry exceptional salary and start-up packages.

The University of Texas at Austin has begun a major new expansion of the Institute for Neuroscience, building upon a strong existing faculty base in Neurobiology, Psychology, Behavior, Pharmacy, Computer Science, Biomedical Engineering, Physics, Chemistry, and the Institute of Cell and Molecular Biology. Successful candidates will have their laboratories in the new Neural and Molecular Sciences Building located within the heart of campus.

Austin is located in the Texas hill country and is widely recognized as one of America's most beautiful and livable cities.

Please send curriculum vitae, summary of research interests, and names of five references to:

Dr. Daniel Johnston, Director
Center for Learning and Memory
Institute for Neuroscience
The University of Texas at Austin
1 University Station, C7000
Austin, TX 78712-0805

Homepage: **http://www.utexas.edu/neuroscience/**

The University of Texas at Austin is an Equal Opportunity Employer. Qualified women and minorities are encouraged to apply; a background check will be conducted on applicants selected.

Neuron

Neuron is seeking an additional full-time scientific editor to join its editorial team based in Cambridge, Massachusetts. To complement the range of expertise on the current *Neuron* team; we are specifically seeking an editor with systems and/or cognitive neuroscience training.

Now in its seventeenth year of publication, *Neuron* publishes the most exciting developments in developmental, molecular, cellular, systems and cognitive neuroscience. The minimum qualification for this position is a PhD in a relevant area of biomedical research, although previous editorial experience is beneficial. This is a superb opportunity for a talented individual to play a critical role in the research community away from the bench.

As a scientific editor, you would be responsible for assessing submitted research manuscripts, overseeing the review process, and commissioning and editing review material for the journal. You would also travel frequently to scientific conferences to follow developments in research and to establish and maintain close ties with the scientific community. The key qualities we are looking for are breadth of scientific interest and the ability to think critically about a wide range of scientific issues. The successful candidate will also be highly motivated and creative, possess strong communication skills and be able to both work independently and as part of a team.

This is a full-time in-house editorial position, based at Cell Press headquarters in Cambridge, Massachusetts. Cell Press offers an attractive salary and benefits package and a stimulating work environment. Applications will be held in the strictest of confidence and will be considered on an ongoing basis. To apply, please email a letter describing your background, interests, and a candid appraisal of the strengths and weaknesses of Neuron, along with your C.V. to **hrma@elsevier.com**. **Please note "Neuron Scientific Editor" in the subject line of your email.** Please, no phone inquiries.

Cell Press is an Equal Opportunity/Affirmative Action Employer; M/F/D/V.

NEUROSCIENCE CAREERS

Faculty Position in Molecular/Cellular Neuroscience

Washington University in St. Louis

Department of Anatomy and Neurobiology

Two faculty positions at Washington University School of Medicine in St. Louis, MO are available for individuals taking innovative approaches to fundamental questions in molecular neuroscience. These positions are in the Department of Anatomy and Neurobiology (**http://thalamus.wustl.edu/**) and will be at the Assistant Professor or Associate Professor level. The department houses 20 active research labs in neurobiology, and is part of a much larger inter-departmental neuroscience program (program website **http://neuroscience.wustl.edu/**). Excellent shared facilities are available for molecular and cellular neuroscience, including imaging (electron and optical microscopy) and mouse genetics (generation and behavioral analysis of transgenic and knockout lines). Both the department and the neuroscience program offer numerous opportunities for scientific interactions and collaborations.

To apply: Send by email attachment **one PDF or Word document** that includes your cover letter, CV, research summary, and names and email addresses of three references. **Send one document only**, limited to 10 pages to **susan@brainvis.wustl.edu**. In addition, please arrange for three letters of recommendation to be sent to **Dr. David Van Essen**, via email to **susan@brainvis.wustl.edu**. Applications and letters must be received by **December 1, 2005**.

AA/EOE M/F/D/V.

MILLER SCHOOL OF MEDICINE
UNIVERSITY OF MIAMI

FACULTY POSITION MOLECULAR AND CELLULAR PHARMACOLOGY

The Department of Molecular and Cellular Pharmacology at the University of Miami Miller School of Medicine is seeking applications for a **TENURE-TRACK FACULTY POSITION** (rank open). Candidates must have a Ph.D. and/or M.D. degree and have an established record of research excellence. Applicants from all areas of molecular/cellular biology and biomedical research are welcome, but we are particularly interested in research relating to the nervous system. The new faculty member will complement existing research efforts in the Department and will have the opportunity to participate in a University-wide Neuroscience program. Rank and salary will be commensurate with experience. Generous laboratory space and start-up funds are available.

Applicants should send electronic and hard copies of their CV, statement of research interests and directions, and contact information for three references, to **ELalor@med.miami.edu** (e-copies) and **Dr. Charles W. Luetje, Search Committee Chair, Department of Molecular and Cellular Pharmacology, University of Miami Miller School of Medicine, P.O. Box 016189, Miami, FL 33101**.

An Equal Opportunity/Affirmative Action Employer.

NEUROSCIENCE CAREERS

MEDICAL COLLEGE OF WISCONSIN

Tenure Track Faculty Position in Neurobiology Department of Cell Biology, Neurobiology and Anatomy

A tenure track faculty position at the Assistant or Associate Professor level is available for a neurobiologist who uses molecular-genetic approaches to address fundamental research questions in a key area of neurobiology. Competitive salary, laboratory space and start-up funds will be provided. Modern facilities include core laboratories for generation of transgenic mice and confocal microscopy. Current research strengths in the department include cellular neurobiology of pain, cellular biology of photoreceptors, molecular neurobiology of mitochondria, and developmental biology of the gastrointestinal, cardiovascular and visual systems. Candidates whose work complements these areas and utilizes state-of-the-art imaging or molecular approaches are especially encouraged to apply.

Contribution to a team taught course in Integrated Medical Neuroscience for medical students and participation in our interdisciplinary graduate program will be expected. Applicants for Assistant Professor must have a Ph.D. or M.D./Ph.D. degree (or equivalent) plus 2 years of postdoctoral experience. Candidates at the Associate Professor level are expected to have established a vigorous, productive and NIH-funded research program. Interested individuals should send a curriculum vitae, statement of research plans, three relevant publications and the names of three references to:

Dr. Cheryl L. Stucky
Chairman, Search Committee
Department of Cell Biology, Neurobiology and Anatomy
Medical College of Wisconsin
8701 Watertown Plank Road
Milwaukee, Wisconsin 53226-0509

For more information visit our Website at:
http://www.mcw.edu/cellbio/

AA/EOE

POSITIONS OPEN

Dental and Craniofacial Scientists

Stony Brook University's School of Dental Medicine seeks tenure or tenure-track faculty at Assistant/Associate/Full Professor rank.

We seek individuals with D.D.S./D.M.D., Ph.D., or M.D. and postdoctoral training. The positions are highly competitive with regard to salary, startup funds, and laboratory space. Successful applicants are expected to generate NIH support for research areas pertinent to dental medicine including but not limited to Stem Cell Biology, Tissue Engineering, Bone Biology, Oral Mucosal Immunity, Keratinocyte Biology, Developmental Biology, Salivary Gland Biology, etc. Preference will be given to individuals with current NIH support.

Academic rank and salary are commensurate with experience and qualifications.

Please submit a letter of application with curriculum vitae, statement of accomplishments and research plans, and names and addresses of three references to:
Maureen Burns, Executive Assistant Dean
School of Dental Medicine
Stony Brook University, SUNY
Stony Brook, NY 11794-8700

AA/EOE. Visit *www.stonybrook.edu/cjo* for employment information.

STONY BROOK

POSITIONS OPEN

Center for Photochemical Sciences
BOWLING GREEN STATE UNIVERSITY
LIGHTING THE WAY

Research Initiative in Photochemical Sciences/Biosciences

The **Center for Photochemical Sciences** in conjunction with the **Departments of Biological Sciences** and **Chemistry at Bowling Green State University** announce the creation of a multidepartmental research initiative at the interface of the chemical, biological, and photosciences. Faculty will be hired in both departments to create a synergistic research cluster to develop and apply advanced phototechniques to study the structure, function, dynamics, and interactions of biomolecules and cellular processes. Such techniques can include, but are not restricted to, single molecule methods or imaging techniques. Creation of this cluster will initially involve one senior and two junior faculty hires as described below. Future expansion is anticipated.

The **Department of Chemistry** invites applicants and nominations for the position of **Ohio Board of Regents Eminent Scholar in Photochemical Sciences**. The successful candidate for this endowed senior professorship will have an established record of research excellence and will be expected to lead the growth and development of the Initiative. The Department provides a stimulating research environment and houses the Ohio Laboratory for Kinetic Spectrometry which maintains modern transient spectroscopy facilities operating within the femtosecond to microsecond time domains, and the Wright Photosciences Laboratory which provides unique opportunities for interactions with industrial photoscientists. See **http://www.bgsu.edu/departments/chem/**. Applications and nominations should be sent to the address below.

The **Department of Biological Sciences** invites applications for two tenure-track positions at the rank of Assistant Professor. The successful candidates will have a demonstrated record of productivity in the above mentioned areas and will be expected to develop strong, extramurally funded research programs in microbial, cellular, or molecular biology. We especially invite enquiries from individuals conducting research having applications in the field of microbiology, in particular, environmental or pathogenic microbiology. The successful candidates will help develop the Photochemical Sciences/Biosciences Initiative and will be members of the Center for Photochemical Sciences. See **http://www.bgsu.edu/departments/biology/**. Interested candidates for these positions should send a copy of their CV, statement of research and teaching interests, and 3 letters of recommendation to the address below.

Applications and nominations should be sent to: **Professor Michael A. J. Rodgers, Ohio Eminent Scholar and Chair of the Faculty Search Committee, Center for Photochemical Sciences, Bowling Green State University, Bowling Green, OH 43403**. Review of applications will begin **December 5, 2005** and continue until the positions are filled.

BGSU is an Affirmative Action/Equal Employment Employer and encourages applications from women, minorities, veterans and individuals with disabilities.

NEUROSCIENCE CAREERS

TWO postdoctoral training fellowships in developmental psychobiology and neurobiology are immediately available and others will become available periodically at the University of Illinois at Urbana-Champaign. This is a National Institute on Child Health and Human Development grant that provides stipends, some research and study funds, and scientific meeting travel to successful candidates. Only U.S. citizens and permanent residents are eligible for support. Minority applicants are especially encouraged to apply. Core faculty mentors include Drs. Chiba, Clayton, Cox, George, Gillette, Gold, Greenough, Hing, Juraska, Korol, Robinson, Roy and Wheeler and applicants interested in other University of Illinois faculty in the area of developmental psychobiology and neurobiology are also invited to apply. Trainees and their mentors are expected to participate in a weekly seminar and monthly meetings associated with the training grant. To apply, submit (1) a one-page letter of application summarizing previous research and future research interests (indicate which faculty member(s) you may wish to work with); (2) a copy of your CV; and (3) three letters of recommendation to: **William T. Greenough, Ph.D., Program Director, University of Illinois, Beckman Institute, 405 North Mathews Avenue, Urbana, IL 61801; wgreenou@uiuc.edu; http://www.life.uiuc.edu/dnptg**, to view faculty research descriptions/profiles. While additional applications may be considered after that date, the initial deadline for applications for the currently available positions is **December 2, 2005**. Please share this notice with your colleagues at other institutions.

The University of Illinois at Urbana-Champaign is an Affirmative Action/Equal Opportunity Employer.

The **Department of Psychological and Brain Sciences** at **Dartmouth College** is seeking applicants for a senior faculty appointment in cognitive neuroscience and to be Director of the Center for Cognitive Neuroscience. The successful candidate is expected to have a distinguished record of accomplishments, including a demonstrated commitment to training students and postdoctoral fellows, as well as a strong potential for sustained external funding. Applications representing any sub-specialization in cognitive neuroscience, broadly defined, are welcome. We are particularly interested in applicants who not only complement our current strengths in memory, cognition, perception and human functional brain imaging, but also make connections to other departmental faculty. The department is housed in a state-of-the-art research and teaching facility that includes a dedicated research MRI scanner for brain mapping research.

Please send a letter of application and a curriculum vitae to: **Dr. Scott Grafton, Chair, Cognitive Neuroscience Search Committee, Department of Psychological and Brain Science, 6207 Moore Hall, Dartmouth College, Hanover, NH 03755**. Informal inquiries regarding the position are also welcome (**scott.grafton@dartmouth.edu**). Review of applications will begin **December 1, 2005** and continue until the position is filled.

With an even distribution of male and female students and over a quarter of the undergraduate student population members of minority groups, Dartmouth is committed to diversity and encourages applications from women and minorities. Dartmouth College is an Equal Opportunity, Affirmative Action Employer.

POSITIONS OPEN

Keratinocyte Biologist

Stony Brook University's School of Dental Medicine seeks tenure or tenure-track faculty at Assistant/Associate Professor rank.

We seek individuals with D.D.S./D.M.D., Ph.D., or M.D. and postdoctoral training. The position is highly competitive with regard to salary, startup funds, and laboratory space.

Successful applicants are expected to generate NIH support for research areas pertinent to epithelial biology including Stem Cell Biology, Keratinocyte Cell and Molecular Biology, or Host Immune Response and Gene Therapy. Preference will be given to individuals with in-vivo as well as in-vitro models. Academic rank and salary are commensurate with experience and qualifications.

Please submit a letter of application with curriculum vitae, statement of accomplishments and research plans, and names and addresses of three references to:
Maureen Burns, Executive Assistant Dean
School of Dental Medicine
Stony Brook University, SUNY
Stony Brook, NY 11794-8700

AA/EOE. Visit *www.stonybrook.edu/jobs* for employment information.

STONY BROOK

Positions @ NIH
THE NATIONAL INSTITUTES OF HEALTH

National Institute of General Medical Sciences

The National Institute of General Medical Sciences (NIGMS) in Bethesda, MD is seeking applications from outstanding candidates for a Health Scientist Administrator (HSA) position in the Pharmacological and Physiological Sciences Branch within the Pharmacology, Physiology, and Biological Chemistry Division. The recruiting branch currently supports research and training into understanding the basis of traumatic and burn injury and the perioperative period, the molecular basis of action of anesthetics, the mechanisms of and genetics underlying the actions of therapeutic drugs, and the development of predictive preclinical toxicology approaches.

The individual hired will be responsible for applying his/her clinical and research expertise to manage and develop research and training grants in NIGMS' broad areas of basic studies in pharmacological and physiological sciences, and to foster the translation of results from fundamental research areas into clinical studies. The person should have experience gained in a medical research institution and understand how research is conducted with human subjects or patients in a clinical setting. A background in at least one of the following areas is preferred: trauma, injury and recovery, or clinical pharmacology, or immune system biology, or alternatively in a cross-cutting area such as studies of the role of inflammation in the disease process or of molecular/cellular signaling in these systems. Experience in modern methods of genome or proteome analysis would also be desirable.

Applicants must possess an MD and/or PhD plus scientific knowledge in the fields of pharmacology, physiology, immunology, systems biology, medicine, or related fields. Applicants must be familiar with both clinical and laboratory approaches in his/her own field(s) of expertise. Experience in the NIH peer review and grant award process would be beneficial. Salary will be commensurate with qualifications, may include a physician's comparability allowance, and will have a full package of benefits. A detailed vacancy announcement **NIGMS-05-100271** with the qualifications and application procedures is available at the NIGMS web page at **http://www.nigms.nih.gov/about/job_vacancies.html**. Questions about application procedures may be directed to **Erin Bandak at 301-594-2324**. Applications must be received by **January 4, 2006**.

Research Fellowship Opening

A Research Fellowship position is available in the Laboratory of Allergic Diseases (LAD), National Institute of Allergy & Infectious Diseases (NIAID) at the National Institutes of Health (NIH) within the Department of Health & Human Services (DHHS) to study receptor mediated signaling pathways in human and rodent mast cells. Utilizing knock out mice, shRNA/siRNA approaches and site directed mutagenesis, studies will focus on how regulatory enzymes and adaptor molecules control gene transcription for the production of cytokines and chemokines. The ideal candidate should have a Ph.D. in molecular biology, immunology, or cell biology, prior postdoctoral experience, and appropriate publication record. Interested individuals should Email or send a cover letter, curriculum vitae, and names and contact details of three referees to:

Alasdair M. Gilfillan Ph.D.
LAD/NIAID/NIH
Bldg. 10, Rm. 11C206
10 Center Drive
Bethesda, MD 20892-1881 USA
Email: agilfillan@niaid.nih.gov

For further details of the laboratory, please visit the web page at: **http://www.niaid.nih.gov/dir/labs/lad/gilfillan.htm**

NIEHS National Institute of Environmental Health Sciences

Tenure-Track Position in Clinical Neurobiology

The Laboratory of Neurobiology in the Division of Intramural Research at NIEHS is recruiting a Tenure-Track Clinical Investigator to establish a high-quality, independent research program on clinical aspects of neurological sciences and disease. Ideally, studies would be conducted on the identification and prevention of environmental disruption of human cognitive potential at any life stage, including early development, childhood learning, or neurodegenerative processes associated with aging. To be considered applicants must have an M.D. or a Ph.D. degree or both, a clinically focused research proposal, postdoctoral experience in clinical research, and a strong publication record. Applicants combining clinical or epidemiological studies with more basic laboratory studies of molecular and cellular aspects of neurobiology, including work with model organisms, are particularly encouraged to apply. The NIEHS has state-of-the-art core facilities for research and an outstanding cadre of epidemiologists and biostatisticians. Excellent start-up funds, salary, and benefits package will be provided. Interested persons should send their curriculum vita with a statement of research accomplishments and plans, and arrange for three letters of recommendation to be submitted to the address below. For information concerning the Laboratory of Neurobiology, access website **http://dir.niehs.nih.gov/dirln/**. For general information about this position, contact **Perry J. Blackshear, M.D., D.Phil., Search Committee Chair, at black009@niehs.nih.gov**. Applications received by **29 December 2005** will be given first consideration. Applications received after that date will be considered only if the position has not been filled.

Applications and letters should be sent to:
Ms. Cindy Garrard (DIR 05-12), National Institute of Environmental Health Sciences, P.O. Box 12233, Maildrop A2-06, 111 Alexander Drive, Room A206, Research Triangle Park, NC 27709 E-mail: dir-appls@niehs.nih.gov

National Human Genome Research Institute

Investigator Recruitment in Cancer Genetics
National Human Genome Research Institute

The Cancer Genetics Branch (CGB) of the National Human Genome Research Institute (NHGRI) is seeking to recruit outstanding tenure-track investigators to pursue innovative, independent research in cancer genetics. General areas of interest include, but are not limited to:

- Cancer gene discover
- Comparative cancer genomics
- Cancer in model organisms
- Cancer proteomics
- Genetic epidemiology
- Cancer pharmacogenomics
- Molecular profiling in tumors
- Functional genomics of cancer
- Genomic instability in cancer
- Markers for early detection

The successful candidate will be able to take advantage of interactions with a highly collegial group of scientists within NHGRI and the NIH campus as a whole. In addition, the successful candidate will have access to NHGRI's outstanding core laboratories.

Candidates must have a Ph.D., M.D., or equivalent degree, as well as comprehensive, advanced training and a record of accomplishment in one of the targeted areas. This position includes a generous start-up allowance, an ongoing commitment of research space, laboratory resources, and positions for personnel and trainees.

Interested applicants should submit a curriculum vitae, a three- page description of proposed research, and three letters of recommendation through our online application system at **http://research.nhgri.nih.gov/apply**. The closing date is **December 1, 2005.**

For more information on CGB and NHGRI's Intramural Program, please see **http://www.genome.gov/Research**. Specific questions regarding the recruitment may be directed to **Dr. Paul Meltzer, the Search Chair, at pmeltzer@mail.nih.gov or by fax (301-480-3281)**. Questions may also be directed to **Dr. Elaine Ostrander, the CGB Branch Chief, at costrand@mail.nih.gov**

Health Scientist Administrator

The National Institute of Dental and Craniofacial Research (NIDCR), National Institutes of Health (NIH), Department of Health & Human Services (DHHS) is seeking applicants for a Health Scientist Administrator position in the Center for Biotechnology and Innovation (CBI). The position is for a Director of the Applied and Translational Research Program. This program emphasizes interdisciplinary-multidisciplinary, highly innovative approach that combines engineering, physics, biology and clinical dental medicine for the restoration/regeneration of orofacial structures (e.g., teeth, bone, salivary glands, periodontal and temporomandibular joint structures, etc.). Relevant areas include: studies regarding the design of bio-inspired new dental composite/ceramic materials through biomimetic principles, a systems approach to the design and development of new biocompatible/interactive materials that can stimulate cells and tissues to regenerate and/or materials that can become integrated into the body; use of stem cells and bioinformatics approaches in regenerating soft and hard tissues/structures of the craniofacial region; computational methods for multiple scaffold designs that can promote stem cell assembly into multi-dimensional structures; design and development of integrated microfluidic platforms based on multiple separation and detection technologies on a single chip in order to obtain inexpensive, rapid detection technologies for biological processes in health and disease; development of delivery vehicles (nanoparticles, artificial matrices) and development of micro-environments where cells can be precisely placed, manipulated and then analyzed in real time. The incumbent will direct, administer and evaluate a portfolio of extramural grants, contracts and cooperative agreements and will stimulate interest in and provide advice to the extramural community regarding the respective portfolio. In addition, the incumbent will participate in funding decisions, policy development, as well as implementation and coordination with other programs both within and outside of the NIDCR. The applicant is required to have a D.D.S., D.M.D., M.D., Ph.D. (or equivalent doctoral degree). The salary range for this position is $88,369 to $114,882 per annum, commensurate with experience. This position has knowledges, skills and abilities (KSA) that must be addressed in order for applicants to be considered. The full vacancy announcement can be viewed at www.usajobs.opm.gov NIDCR-05-966604. Applications will be accepted until **November 21, 2005**. Please submit materials to: **Elan Ey, Branch I, Office of Human Resources, NIH, 6707 Democracy Blvd., Suite 400, Bethesda, MD 20892-5482 or by email: elan.ey@nih.gov**. U.S. Citizenship is required.

National Human Genome Research Institute

Postdoctoral Fellowship
in Vertebrate Genetics and Developmental Biology

The Developmental Genetics Section, Genetic Disease Research Branch of the National Human Genome Research Institute is seeking an outstanding postdoctoral fellow to study the molecular control of mammalian embryonic development and oncogenesis. The lab is particularly interested in the roles of Wnt and Hedgehog signaling pathways in pattern formation and skeletal morphogenesis using combined approaches of mouse genetics, cell biology and genomics (http://www.genome.gov/Staff/Yang).

Candidates must have an M.D. and/or Ph.D. degree and less than 5 years of postdoctoral experience. Candidates must also have a strong background in molecular genetics, cell biology and developmental biology, as well as a record of relevant peer-reviewed publications, the ability to speak and write effectively, and be self-motivated and creative. Interested applicants should submit a curriculum vitae and a statement of research interest to **Dr. Yingzi Yang, NHGRI/NIH, 49 Convent Drive, Building 49, Room 4A68, Bethesda, MD 20892 or e-mail to: yyang@nhgri.nih.gov.**

UMDNJ-ROBERT WOOD JOHNSON MEDICAL SCHOOL & THE CHILD HEALTH INSTITUTE OF NEW JERSEY

FACULTY SEARCH

The Child Health Institute of New Jersey (CHINJ) at UMDNJ-Robert Wood Johnson Medical School (RWJMS) is launching a major research initiative in developmental biology to be housed in its new research facility. We are searching for several outstanding candidates with an emerging or established program in the field of vertebrate developmental biology who utilize model organisms to gain fundamental insights into congenital malformations and developmental disabilities. Areas of particular interest include, but are not limited to, molecular and cellular mechanisms of organogenesis, epigenetic control of tissue specification, and development of the craniofacial, nervous, and immune systems. Qualified candidates will be considered for independent tenure-track faculty positions at the Assistant, Associate or Full Professor levels. Successful recruits will receive competitive start-up packages and will have faculty appointments at UMDNJ-RWJMS with full access to graduate training programs.

CHINJ is located in the New Brunswick campus and includes 40,000 sq. ft. of laboratory and office space and a 25,000 sq. ft. mouse barrier facility, in addition to shared core laboratories for mouse gene targeting and transgenesis, histology/pathology, in vivo imaging, optical microscopy, and microarray analysis. The Institute program will complement the clinical program of the Bristol-Myers Squibb Children's Hospital to create an international center of excellence in pediatric care and biomedical research. It will also interface with germane initiatives at RWJMS and Rutgers University, including those of the neighboring Cancer Institute of New Jersey and soon-to-be-established Cardiovascular Institute of New Jersey and Stem Cell Institute of New Jersey.

Robert Wood Johnson Medical School is one of eight schools of the University of Medicine & Dentistry of New Jersey (UMDNJ). The medical school is dedicated to the pursuit of excellence in the education of health professionals, in the conduct of biomedical, clinical and public health research, in the diversity of health care and in the promotion of community health for residents of the state. With twenty-one basic science and clinical departments and 85 centers and institutes, 2,500 full-time and volunteer faculty, and 600 medical students, the school has active programs on its three campuses in New Brunswick, Piscataway and Camden.

Interested applicants should send a curriculum vitae, a concise description of their research interests, and the names of three (3) references to: **Dr. Michael Shen, Ph.D., Chair, CHINJ Search Committee, Center for Advanced Biotechnology and Medicine, 679 Hoes Lane, Piscataway, NJ 08854-5638.** The University of Medicine & Dentistry of New Jersey is an equal opportunity/affirmative action employer.

UMDNJ ROBERT WOOD JOHNSON MEDICAL SCHOOL
University of Medicine & Dentistry of New Jersey

Child Health Institute of New Jersey

UNIVERSITY OF TOLEDO TENURE-TRACK POSITIONS IN CELL/MOLECULAR BIOLOGY

The Department of Biological Sciences at the University of Toledo is seeking to fill four tenure-track assistant professor faculty positions as part of a major new hiring initiative over the next two years. Departmental research initiatives include cellular immunology, cancer biology, nematode molecular biology, and plant biology. The new positions will enhance existing research strengths. Facilities include a modern research complex with state-of-the-art laboratories and outstanding instrumentation centers with plans underway for a new science building. Applicants must have a Ph.D. and postdoctoral experience. Successful candidates should have or will be expected to develop an externally funded research program and will participate in undergraduate and graduate instruction. The Department offers the B.S., M.S., and Ph.D. degrees. Additional information is available on the departmental web site at **www.biosciences.utoledo.edu**. Salary and set-up funds are competitive.

Review of applications will begin **November 28, 2005** and continue until the positions are filled. The starting date for three of these positions will be August 2006.

Interested candidates should send a letter of application, curriculum vitae, statements of teaching and research interests, and arrange to have three letters of recommendation sent to: **Chair, Faculty Search Committee, Department of Biological Sciences, MS 601, University of Toledo, Toledo, Ohio 43606-3390.** Email inquiries may be directed to **patricia.komuniecki@utoledo.edu**.

Qualified women and minorities are encouraged to apply. The University of Toledo is an Affirmative Action/Equal Opportunity Employer M/F/D/V.

University of Pennsylvania

Faculty Posititions

Leonard and Madlyn Abramson Family Cancer Research Institute at the University of Pennsylvania

The University of Pennsylvania seeks outstanding candidates for the tenure-track faculty positions in the Abramson Family Cancer Research Institute, which is an integral part of the University of Pennsylvania Abramson Cancer Center and the School of Medicine. Faculty appointment will be tenure-track Assistant, Associate or Full Professor in an appropriate department in the School of Medicine. Rank will be commensurate with experience. Applicants must have an MD and/or PhD degree.

Candidates who have scientific interests and experience in any field of cancer biology, including but not limited to translational research, cancer stem cells, cell cycle control, tumor suppressor genes, oncogenes, cancer genetics, signal transduction, angiogenesis, and apoptosis, should send a letter of interest, curriculum vitae and the names of references to:

Craig Thompson, M.D.
Scientific Director
The Abramson Family Cancer Research Institute
4th Floor – BRB II/III
421 Curie Blvd.
Philadelphia, PA 19104

www.uphs.upenn.edu/abramson

Leonard & Madlyn ABRAMSON family CANCER RESEARCH INSTITUTE at the University of Pennsylvania Cancer Center

The University of Pennsylvania is an equal opportunity/affirmative action Employer. Women and minority candidates are strongly encouraged to apply.

BIOMEDICAL DIAGNOSTICS INSTITUTE

The Biomedical Diagnostics Institute (BDI) was established in October 2005 at Dublin City University, through an award of €16.5M from Science Foundation Ireland (SFI) under its Centres for Science, Engineering and Technology (CSET) programme, in addition to a €6M contribution from industry partners. The BDI will carry out cutting-edge research programmes focused on the development of next-generation biomedical diagnostic devices measuring indicators of chronic disease (e.g. cancer, cardiovascular disease).

We are currently assembling a team of world-class research scientists to partner with cutting-edge research teams from our industry partners (Analog Devices, Amic, Enfer, Hospira, Becton Dickinson & Inverness Medical Innovations) and collaborating institutions (The Royal College of Surgeons Ireland (RCSI) in Dublin, the National Centre for Biomedical Engineering Science (NCBES) at NUI, Galway, and the Tyndall National Institute (TNI) in Cork). The BDI team will be based primarily in DCU, with some researchers located in our collaborating institutions. Some applicants for the Postdoctoral positions may be offered employment by our industrial partners.

Applications are invited for the following contract positions:

RESEARCH PROGRAMMES

RESEARCH FELLOWS, POSTDOCTORAL RESEARCHERS & POSTGRADUATE STUDENTS are invited to apply for various positions in the following programmes:

Biomolecular Recognition (REF: BDI-RP1)
Prof Richard O'Kennedy (richard.okennedy@dcu.ie)

Goal: To develop novel antibody and nucleic acid-based assays and to incorporate them into biochip platforms.

Expertise: Antibody production/engineering and immunoassay development / Nucleic acid-based analysis / Immobilisation and surface chemistry of biomolecules.

Functional Diagnostics in Platelet Biology (REF: BDI-RP2)
Prof Dermot Kenny (dkenny@rcsi.ie)

Goal: To develop novel physiologically relevant assays of platelet function.

Expertise: Cell biology of platelet function & thrombosis / Molecular Protein chemistry / Rheology.

Transduction Science (REF: BDI-RP3)
Prof Robert Forster (robert.forster@dcu.ie)

Goal: To develop sensitive and selective detection strategies for proteins and DNA through combinations of current and light detection.

Expertise: Electrochemiluminescent materials especially luminescent polymers / Interfacial characterisation techniques – Raman and scanning probe microscopy / Electrochemical / luminescent bioassay development.

Signal Amplification Science (REF: BDI-RP4)
Prof Brian MacCraith (brian.maccraith@dcu.ie)

Goal: To develop substantial sensitivity enhancements in a range of optical biochip systems, with the emphasis on fluorescence-based platforms.

Expertise: Metal-enhanced fluorescence – Plasmonics / Optoelectronic readout instrumentation for biochips / High-brightness nanoparticle labels.

Microfluidic Platforms (REF: BDI-RP5)
Prof Luke Lee (luke.lee@dcu.ie) & Prof Tony Ricco (tony.ricco@dcu.ie)

Goal: To develop advanced microfluidic platforms for diagnostic applications.

Expertise: Microfluidics and microfabrication / Integrated detection techniques / Cell biology.

Coagulation Monitoring (REF: BDI-IP1)
Dr Tony Killard (tony.killard@dcu.ie)

Goal: To develop advanced coagulation monitoring devices for chronic and critical care applications (including wearable closed-loop anticoagulant therapy systems).

Expertise: Rheological, viscoelastic and haemostatic properties of blood / Polymer MEMS & microfluidics / Biodevice interfacial modification / Integration of sensors and wireless technology.

Biochip for Cardiac Wellness (REF: BDI-IP2)
Prof Brian MacCraith (brian.maccraith@dcu.ie)

Goal: To develop a multianalyte, capillary-fill biochip for monitoring markers of cardiac wellness.

Expertise: Microfluidics / Optical biosensors based on fluorescence / Immobilisation of biomolecules.

Bovine Mastitis Diagnostics Chip (REF: BDI-IP3)
Prof Richard O'Kennedy (richard.okennedy@dcu.ie)

Goal: To develop a multi-analyte miniaturised assay platform for the detection of mastitis.

Expertise: Immuno / Nucleic Acid-based assay development and validation / Analyte extraction & sample preparation / Microbiological and Biochemical analysis.

TECHNICIANS (REF: BDI-TN)

Technicians are also required to support the above research programmes. Applicants must hold a minimum qualification in a relevant discipline at National Certificate and preferably at National Diploma level, or equivalent.

Salary: €30,626 – €50,137

MANAGEMENT & ADMINISTRATION

INSTITUTE MANAGER (REF: BDI-MN-MNG)

The Manager will support the Director in the establishment and ongoing development of the Institute and will facilitate the achievement of the Institute's objectives by the effective management of its resources and operations, including financial and administrative issues. In addition, the Institute Manager will be responsible for all aspects of the Institute's business relations and commercialisation. Applicants must have an honours degree with at least three years postgraduate experience of team management.

Salary: €72,864 – €96,039

Queries To: Prof Brian MacCraith (brian.maccraith@dcu.ie)

EDUCATION & OUTREACH (E&O) PROGRAMME

The BDI has designed an ambitious E&O programme with initiatives targeting the general public and all levels of education from primary through to post-graduate level. Applications are invited for the following positions:

EDUCATION & OUTREACH MANAGER (REF: BDI-EO-MNG)

Reporting directly to the E&O Leader, the E&O Manager will have responsibility for the development and implementation of the Institute's E&O programme. He/she will also manage the overall science communication function of the BDI. Applicants must have a minimum of an honours science / engineering degree with three years relevant postgraduate experience.

Salary: €46,334 – €67,607

POSTDOCTORAL RESEARCHERS, RESEARCH ASSISTANTS & POSTGRADUATE STUDENTS (REF: BDI-EO-RES)

Postdoctoral Researchers, Research Assistants and Postgraduate Students are required for the development and delivery of the E&O programme. We are seeking candidates with expertise in one or more of the following areas:

- Science Education or Science Communication
- Lecturing experience in Biomedical Diagnostics
- Development & co-ordination of Masters Degree programmes

Queries To : Prof Richard O'Kennedy (richard.okennedy@dcu.ie)

APPLICATION PROCEDURES:

Applicants are encouraged to contact the appropriate principal investigator for informal discussions.

Applications (complete Application Form & Curriculum Vitae) should be marked with the appropriate reference code and submitted to:
Human Resources Department, Dublin City University, Dublin 9, Ireland.

Application forms are available from: ***Human Resources Department, Dublin City University, Dublin 9, Ireland.***
Tel: ***+353 (0) 1 700 5149*** *Fax:* ***+353 (0) 1 700 5500*** *Email:* ***hr.applications@dcu.ie***

Full job descriptions are available at: ***http://www.dcu.ie/vacancies/current.shtml***

Closing date for receipt of applications: ***Friday 25th November, 2005***

science foundation ireland
fondúireacht eolaíochta éireann

NATIONAL DEVELOPMENT PLAN

FELLOWSHIPS

International Leibniz Research School for Microbial and Molecular Interactions (ILRS Jena)

At the Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knoell-Institute - in co-operation with the Friedrich-Schiller-University Jena and the Max Planck Institute for Chemical Ecology

15 fellowships for PhD Students

are available.

The program starting February 1st 2006 will cover the following topics:
- interactions between microorganisms
- host/microbial pathogen interactions
- role of networks and bioinformatics

and is open to outstanding graduates with a master´s degree, diploma or equivalent in biology, biochemistry, pharmacy, bioinformatics and other life sciences.

Jena is an upcoming research area which offers an exciting environment and access to state-of-the-art research facilities.

The program consists of a three-year experimental project combined with an ambitious graduate curriculum. Communication and teaching language is English. Applicants are invited to visit our website **www.ilrs.hki-jena.de** and to identify 3 projects of interest.

Candidates should send an informative letter of interest (300 words in maximum) by e-mail to Prof. Dr. Axel Brakhage (address below). Deadline is **November 20th 2005**. Selected applicants will be called to send complete applications and will get detailed information about the recruitment process.

For further information please contact:

Prof. Dr. Axel Brakhage
Leibniz Institute for Natural Product Research and Infection Biology
- Hans-Knoell-Institute -
and Friedrich-Schiller-University Jena
Beutenbergstrasse 11a, 07745 Jena, Germany
ilrs@hki-jena.de

Yale University
School of Forestry & Environmental Studies

TROPICAL RESOURCES SCIENTIST

A tenured faculty position is available for a tropical resources scientist at the Yale School of Forestry and Environmental Studies. Candidates will have a Ph.D., strong grounding in natural resource sciences with an active, applied research program in the tropics and active engagement in the policy process. We seek, especially, candidates with an interest in research issues at the interface between natural and social sciences, human-impacted ecosystems, or the role of humans in managing tropical forest resources.

Applicants should send, by **November 30, 2005**, their curriculum vitae, statements of research and teaching interests, a list of three references, and three representative publications to **David Skelly, Chair, Tropical Resources Scientist Search, 205 Prospect St., School of Forestry and Environmental Studies, New Haven, Connecticut 06511.** For additional information, please contact **David Skelly (david.skelly@yale.edu).**

Yale University is an Equal Opportunity/ Affirmative Action Employer. Women and minority scholars are encouraged to apply.

University at Buffalo
The State University of New York

Tenure-Track Position in Bacterial Pathogenesis

Job Description: A tenure-track position at the assistant, associate, or full professor level is available in the School of Medicine and Biomedical Sciences for an outstanding scientist with research interests related to bacterial pathogenesis. Although junior candidates are preferred, more senior candidates having appropriate accomplishments will also be considered. An attractive start-up package is available. Individuals with expertise in genetics, molecular pathogenesis, host-pathogen interactions, or infectious disease are encouraged to apply, as are those individuals who employ modern approaches such as bioinformatics, proteomics, or genomics. The ideal candidate will have a doctoral degree and at least two years of post-doctoral research experience in bacterial pathogenesis. He/she will be expected to establish and maintain an extramurally funded research program and to contribute to the graduate and professional teaching responsibilities of the department. The Department of Microbiology and Immunology is strongly research-oriented, with major programs in bacteriology, parasitology, virology, and immunology. Most departmental faculty are members of The Witebsky Center for Microbial Pathogenesis and Immunology, an interdisciplinary group housed within a state-of-the-art biomedical research building. For more details on faculty and research programs, refer to: <www.smbs.buffalo.edu/microb> and <www.smbs.buffalo.edu/wcmpi>.
The University at Buffalo (UB) has Schools of Medicine, Dental Medicine, Public Health, and Pharmacy. The Hauptmann-Woodward Institute (HWI), an internationally recognized structural biology institute, is located near the university. A new research building with 400,000 sq.ft of research space for housing The New York State Center for Bioinformatics and Life Sciences, shared jointly by UB and The Roswell Park Cancer Institute, is scheduled to open in Spring 2006.
Applications received before March 31, 2006 will be given preference. Applications from minorities and women are especially encouraged.

Candidates should submit (1) a curriculum vitae, (2) reprints of their two most important papers, (3) names and contact information for three referees, and (4) a description of present and future research plans to:

Dr. J. Hay
Department of Microbiology and Immunology
138 Farber Hall, UB SMBS
3435 Main Street, Buffalo NY 14214
E-mail: jhay@buffalo.edu

An Affirmative Action / Equal Opportunity Employer

POSTDOCTORAL RESEARCH FELLOWSHIP
Department *of* Immunology

BCM Baylor College of Medicine

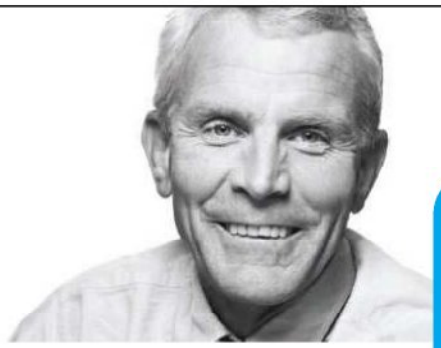
Applications are now being accepted from recent graduates for postdoctoral research fellowship positions in the Department of Immunology at Baylor College of Medicine, through the NIH Training Grant, "Molecular and Cellular Mechanisms of Host Defense." Located in Houston TX, Baylor College of Medicine is a leader in biomedical research. The program provides mentorship in individual career development, curriculum in grant writing and presentation skills, as well as research opportunities in critical areas of immunology: signal transduction, lymphocyte development, HIV pathogenesis, apoptosis, autoimmunity, gene therapy and genetic vaccines.

Applicants must be recent graduates, as well as United States citizens or permanent residents. To apply, send a statement of research interest and eligibility, C.V., and the names and email addresses of three references to:

Tse-Hua Tan, Ph.D., Professor, Baylor College of Medicine
c/o rmcuthbe@bcm.edu

Immunology Training Faculty:
Michael A. Barry, Ph.D.
John W. Belmont, M.D., Ph.D.
Si-Yi Chen, M.D., Ph.D.
David Corry, M.D.
Farrah Kheradmand, M.D.
Margaret Goodell, Ph.D.
Shuhua Han, M.D.
David P. Huston, M.D.
Dorothy E. Lewis, Ph.D.
Cliona Rooney, Ph.D.
C. Wayne Smith, M.D.
David M. Spencer, Ph.D.
Tse-Hua Tan, Ph.D.
Jin Wang, Ph.D.
Rongfu Wang, Ph.D.
Li-Yuan Yu-Lee, Ph.D.
Biao Zheng, M.D., Ph.D.

www.bcm.edu/Immuno
BCM is an equal EEOC/AA/EA employer



» I believe that it is actually within the realm of the possible that I can be a part of preventing diabetes. I think that is incredible! «

Anders Dejgaard Dr. Med., Vice President

Head of Cancer Research

Novo Nordisk has recently decided to establish a dedicated Biopharmaceuticals Research Unit and increase efforts in the area of cancer research. As part of the expansion we have established a Cancer Pharmacology department. We are now seeking an excellent individual to lead the department and take responsibility for furnishing it to support the overall strategy for the cancer research area.

Challenges: We are seeking a highly motivated and skilled individual to lead and expand the team that presently includes three PhDs and six lab. technicians. A rich pre-clinical project pipeline is in place, and several highly innovative cancer projects are in development. You will be directly involved in the project work, contributing personally to the innovation and successful flow of the project pipeline. We wish to increase the staff and introduce new state-of-the-art technologies over the coming years, in order to fully match the project needs as the pipeline expands. Technology will also be accessed through external collaboration; the head of department will be pivotal in pursuing such activities with the team. The focus of Cancer Pharmacology is on in vivo models for pharmacological investigations of new biological entities. Relevant supporting technologies, i.e cell biology, histology and pharmacokinetics, are available within the department as well as in other departments in the research unit.

Qualifications: The position requires a strong scientific background (MD or PhD and several years of postdoc experience) in oncology and expertise in mechanistic studies in cancer models. Proficiency in in vivo handling procedures such as experience with anaesthesia and surgery is required. Leadership talent and excellent communication skills are important, and previous experience from pharma industry or biotech is preferred. You are a strong team-player who will support and further develop the winning culture spirit in our organisation.

Contact: For further information, please contact Ian Ahnfelt-Rønne, Vice President and Head of Pharmacology at +45 4443 4541.

Please forward your application online marked "NN21357-K Head of Cancer Research Department".

novonordisk.com/job

Being there: With more than 20,000 employees in 69 countries, diversity is a key word at Novo Nordisk. Innovation, passion and professionalism are qualities that unite us across national borders and guide us towards our goal of being the best in our markets and making a significant difference to people within the areas of diabetes, growth hormone therapy, haemostasis management and hormone replacement therapy. Our turnover in 2004 was 29 billion DKK and we market our products in more than 180 countries.

novo nordisk®

CASE
CASE WESTERN RESERVE UNIVERSITY

Department of Pathology, Case Western Reserve University School of Medicine announces recruitment for Assistant, Associate or Full Professors in the following areas:

1. **Basic Research in Cancer Biology**
2. **Translational Research in Cancer Biology**
3. **Research in the Immunobiology of Cancer**

The Department of Pathology at Case Western Reserve University School of Medicine (**http://www.case.edu/med/pathology/index.htm**) is initiating an expansion in the size of the Department's faculty and the scope of its research activities. Much of this expansion will be accommodated in the newly constructed Wolstein Research Building (**http://www.case.edu/visit/tours/health/7.html**), where the Department will occupy approximately 41,000 square feet of research space on the 5th and 6th floors. As part of this initiative, the Department is recruiting biomedical scientists at the ranks of Assistant, Associate or Full Professor to contribute to the Department's research efforts in cancer biology and immunobiology. Candidates must hold the Ph.D. and/or M.D., and/or D.V.M degree, or their equivalents. Candidates with strong track records of research creativity, productivity, and extramural funding are sought in the following three areas:

Basic research in the field of cancer. We are seeking candidates whose work may involve the use of model organisms to identify fundamental biological mechanisms with direct or indirect relationship to malignant transformation, invasion and metastasis in mammals, as well as candidates working in human, mouse or other mammalian systems of relevance to cancer.

Translational research in the field of cancer. We are especially seeking candidates whose work emphasizes the development and exploitation of new technologies and strategies in genomics, proteomics, or glycomics to aid in the diagnosis and treatment of cancer. The Department offers an expanding set of opportunities to work with human tissue material via the Department's surgical pathology and clinical pathology faculty.

Immunobiology of cancer. Candidates are sought with strong track records in unveiling new paradigms at the interface between the immune system and malignantly transformed cells, or with a basic immunobiology research focus that will complement research programs in cancer immunobiology.

Competitive startup packages are available for candidates wishing to begin a career at the Assistant Professor rank. Competitive transitional support packages are available for candidates with established research programs at the Assistant Professor, Associate Professor, and Full Professor ranks. The Department offers opportunities to combine research activities with clinical service activities in surgical or clinical pathology. There are abundant opportunities for collaborative research in our Department, others at Case, and in the Case Comprehensive Cancer Center (**http://cancer.case.edu/**) and its core facilities. The Department of Pathology maintains a vigorous Ph.D. program, and is committed to providing its faculty with a strong mentoring environment. The Cleveland metropolitan area offers a vibrant culture and recreational environment, a substantial diversity of city and suburban living opportunities, and an affordable cost of living index (**http://www.cwru.edu/president/aaction/relocation.html**).

Academic rank and salary will be commensurate with qualifications and experience. Send curriculum vitae, a statement outlining the scope of your existing or planned research program and career goals and names of three references to:

John Lowe, M.D.
Henry Willson Payne Professor and Chair, Department of Pathology
Case Western Reserve University
10900 Euclid Avenue
Cleveland, OH 44106-7288

Phone: 216-368-3611
Facsimile: 216-368-1539
Email: John.Lowe@Case.edu

In employment, as in education, Case Western Reserve University is committed to Equal Opportunity and World Class Diversity.

THE UNIVERSITY OF IOWA

FACULTY POSITIONS AVAILABLE

Presidential Biological Scholar Program

The Carver College of Medicine and the College of Liberal Arts and Sciences at the University of Iowa are seeking new investigators of outstanding promise in the basic biological and clinical sciences. Up to four young investigators will be named as Presidential Biological Scholars at the rank of assistant professor in the tenure-track. The award will provide significant financial research support for a period of four years. Funding for salary and appropriate research space will be provided by a departmental appointment. Scholars may apply directly to the Presidential Biological Scholar Program or be nominated by Department Heads of the Carver College of Medicine's basic and clinical academic departments, or by Department Chairs of units in the College of Liberal Arts and Sciences at the University of Iowa.

Candidates must have a Ph.D., or equivalent, and a demonstrated record of excellence in scholarship as evidenced by publications in leading journals in appropriate disciplines. Applications, including a cover letter indicating the faculty position of interest, curriculum vitae, list of references, and a summary of research accomplishments and future plans, can be sent to:

Presidential Biological Scholar Program
Richard Smith, M.D., Chair
William Nauseef, M.D., Co-Chair
Attn: Sonya Housholder
Office of the Dean, 200 CMAB
The University of Iowa
Carver College of Medicine
Iowa City, IA 52242-1101

A list of eligible departments is available at:
http://www.uiowa.edu/~pbschol

The University is an Affirmative Action/Equal Opportunity Employer and strongly encourages applications from women and minority candidates.

EDCTP – European & Developing Countries Clinical Trials Partnership

The mission of the European and Developing Countries Clinical Trials Partnership (EDCTP) is to accelerate the development of new or improved drugs and vaccines against HIV/AIDS, malaria and tuberculosis through the funding of phase II and III clinical trials and capacity strengthening to support high quality scientific research within Africa.

EDCTP provides a unique innovative platform for a better coordination of European and African scientific programmes and involves African partners at each step of its decision making process with the final objective of reducing poverty by improving the health of the world's poorest continent.

Grants awarded and planned Calls

EDCTP has 4 major grant schemes:

1) Clinical Trials:

Calls launched in 2005:

- Capacity Building for the conduct of clinical trials of microbicides and vaccines against HIV (planned for end of 2005) and tuberculosis
- Support of clinical trials for the identification of safe ARV in combination with tuberculosis drugs in tuberculosis patients with HIV infection

Calls planned for 2006:

- Support of clinical trials to prevent mother to child transmission of HIV
- Support of clinical trials on simplified treatment strategies for HIV
- Support of clinical trials for microbicides
- Support clinical trials for the treatment of malaria in pregnant women
- Support capacity building for the conduct of clinical trials for malaria vaccines
- Support of clinical trials for treatment of uncomplicated malaria
- Support of clinical trials for TB vaccines

2) Capacity Building

Calls launched in 2005:

- Capacity building for ethical review in Africa (support of an African coordination office, support for courses or seminars and Support of the establishment of ethical review boards)

3) Training Awards

Annual launch of calls supporting:

- 15 MSc Studentships
- 5 PhD scholarships
- 5 Career Development Awards
- 6 Senior Fellowships

4) Networking

Calls launched in 2005:

- Providing incentives for joint capacity building programmes
- Promotion of networks of training facilities for clinical monitors
- Sponsorship of meetings or workshops of sustainable networks
- Coordination of research activities in Africa
- Support for national African networks of scientists working on the 3 target diseases

For more information about the EDCTP please visit our website at **www.edctp.org** or contact:

The Hague Office
Dr. Cynthia Naus
Programme Coordinator
Tel: +31 (0) 70 3440880
Fax: +31 (0) 70 3440899
Email: naus@edctp.org

Cape Town Office
Dr. Michael Makanga
Capacity Building Manager
Tel: +27 (0) 21 9380509
Fax: +27 (0) 21 9380569
Email: makanga@edctp.org

PROGRAM HEAD, GENETICS & GENOMIC BIOLOGY

The Hospital for Sick Children Research Institute

The Hospital for Sick Children Research Institute is seeking a new Head for its highly successful Genetics and Genomic Biology Program. Researchers in this program have contributed groundbreaking discoveries in human genetics, disease gene discovery, genome structure, comparative genomics, epigenetics and human developmental genetics. The new Program Head will bring an internationally recognized research program in genetic and genomic research that complements and enhances the current program strengths. The new Head will also bring proven leadership and mentorship skills to the program and will play a major role in promoting genetics within the broader mission of the hospital in integrating research, education and clinical care in the quest to improve the health of children.

The Hospital for Sick Children (SickKids), located in Toronto, Ontario, is Canada's most research intensive hospital and the largest centre dedicated to improving children's health in the country. Its mission is to provide the best in family-centred, compassionate care, to lead in scientific and clinical advancement, and to prepare the next generation of leaders in child health. As Canada's leading paediatric academic health sciences centre, SickKids provides access to diverse patient populations for genetic research. The Hospital for Sick Children Research Institute houses a broad base of research programs from fundamental discovery research through to clinical applications and population outcomes in child and youth health. For more information on the research programs visit: **www.sickkids.ca/research.**

The Hospital for Sick Children is fully affiliated with the University of Toronto and is located within the vibrant Toronto biomedical research community. Successful candidates will hold a PhD or MD and will be eligible for a senior level academic appointment at the University of Toronto.

Interested candidates should submit an application, including a curriculum vitae, a summary of research achievements and leadership skills, and the names, addresses, telephone numbers and e-mail addresses of three references, by January 15, 2006, to: **Dr. Janet Rossant, Chief of Research, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8. Fax: (416) 813-5085. E-mail: janet.rossant@sickkids.ca.**

The Hospital for Sick Children and the University of Toronto are strongly committed to diversity within the community and especially welcome applications from visible minority group members, women, Aboriginal persons, persons with disabilities, members of sexual minority groups and others who may contribute to further diversification of ideas. All qualified candidates are encouraged to apply, however, Canadian and permanent residents will be given priority.

SickKids®
TORONTO, CANADA

University of Toronto

SCOTT & WHITE

College of Medicine
The Texas A&M University System Health Science Center

Pediatric Hematology-Oncologist

The Section of Pediatric Hematology/Oncology at **Scott and White Clinic** and the **Texas A&M University System Health Science Center College of Medicine** (TAMUS HSC-COM) are seeking a clinician scientist with current research grants for a faculty position in a rapidly growing program. The candidate should be BE/BC in pediatric oncology and committed to an academic career. The successful candidates will join and enhance ongoing efforts in basic and translational research, with an institutional commitment to building a world-class experimental therapeutics program. An outstanding start-up package includes high quality laboratory space, excellent benefits and competitive salaries commensurate with academic qualifications. The position guarantees 75% protected time for research activities.

Scott & White Clinic is a 500+ physician directed multi-specialty group practice that is the leading provider of cancer care in Central Texas. Scott and White Clinic and the 486 bed tertiary Scott & White Memorial Hospital is the main clinical teaching facility for TAMUS HSC-COM. Outstanding clinical practice and laboratory facilities on campus that perform state of the art molecular and cellular biology research, flow cytometry, genomics and biostatistics are in place to support the research effort.

Please contact: **Don Wilson, M.D. Professor and Chairman, Department of Pediatrics, Scott & White, 2401 S. 31st, Temple, TX 76508. (800)725-3627 dwilson@swmail.sw.org Fax (254) 724-4974.**

For more information about Scott & White, please visit www.sw.org For Texas A&M www.tamhsc.edu. Scott & White is an equal opportunity employer.

MILLERSVILLE UNIVERSITY

ANIMAL DEVELOPMENTAL BIOLOGIST

The Department of Biology at Millersville University invites applications for a tenure-track position at the Assistant Professor level beginning in August 2006 (Fall term). The ideal candidate can: a) teach undergraduate courses in introductory biology, b) teach a Developmental Biology course with laboratory, c) teach an upper level course in an area of specialization, and d) supervise undergraduate research.

Required: Ph.D. in animal/biological sciences with broad (from whole animal to molecular level) training and experience in developmental biology, teaching experience at the undergraduate level, a strong commitment to liberal arts education, good general knowledge of biology, excellent communication skills, record of publication that uses molecular techniques to investigate some aspect of animal development, successful interview and teaching demonstration.

Preferred: Teaching and research experience beyond the doctorate, a record of scholarly presentations at professional meetings, and training and experience that would permit them to contribute to the teaching of Zoology, Cell Biology, Genetics, Molecular Biology, and/or a cellular and molecular techniques course. It is desirable that the candidate has interests that complement existing programs in the department with 19 full-time Ph.D. faculty positions and over 500 undergraduate majors.

The new and renovated science complex contains modern facilities including ultra and high-speed centrifuges, cell culture facilities, fluorescence microscopy, well-equipped molecular biology labs, a scanning electron microscope, plus computer and audiovisual equipment. Additional information can be found at the department's web site: http://muweb.millersville.edu/~biology/index.php.

Full consideration will be given to applications received by **December 21, 2005.** No electronic submissions accepted. To apply, please submit the following items: 1) letter of application addressing qualifications, 2) statement of research and teaching interests, including documentation of previous teaching experience/performance, 3) current curriculum vitae, 4) copies of transcripts, 5) recent published papers and manuscripts in press, and 6) three current letters of reference (at least one of which addresses teaching skill), sent by the referee, to: **Dr. James Moné, Chair, Animal Development Search Committee, Department of Biology/SC1104, Millersville University, P.O. Box 1002, Millersville, PA 17551-0302.**

An EO/AA Institution

UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

School of Molecular & Cellular Biology and College of Medicine

The School of Molecular and Cellular Biology (http://www.life.uiuc.edu/mcb/) and the College of Medicine (http://www.med.uiuc.edu/) at the University of Illinois at Urbana-Champaign invite applications for multiple tenure track faculty positions as described below. The starting date for these positions is August 16, 2006. These positions offer the opportunity to join a rapidly growing group of outstanding biological scientists on a campus that provides a highly interactive, interdisciplinary research environment and state-of-the-art research support facilities. The University of Illinois at Urbana-Champaign has added significant faculty strength in the biological sciences over the last five years and we anticipate additional hires in these and related areas each year for the next several years. Each of these positions offers excellent laboratory facilities, substantial start-up funds, and the opportunity to work with outstanding graduate students.

The UIUC campus offers a wide range of state-of-the-art research support facilities, including mass spectrometry, NMR, X-ray crystallography, micro- and nanoscale fabrication and analysis, the Roy J. Carver Biotechnology Center, the W. M. Keck Center for Comparative and Functional Genomics as well as facilities for proteomics, metabolomics, immunological resources and flow cytometry. Superb resources for computational biology are available on campus at the National Center for Supercomputing Applications and the NIH Resource for Macromolecular Modeling and Bioinformatics. The Institute for Genomic Biology (http://www.igb.uiuc.edu/), a new 186,000 square foot facility devoted to biological research, will open in 2006.

Salaries for these positions are commensurate with experience and are competitive. Urbana-Champaign offers the residential advantages of a medium-sized university city, excellent cultural opportunities, and easy access to Chicago, St. Louis, and Indianapolis.

Biochemistry – Assistant Professor

We invite applications for a full-time, tenure-track faculty position at the Assistant Professor level in the Department of Biochemistry. We are seeking outstanding candidates whose research investigates the molecular basis of biological or biomedical processes. Our priority is for candidates interested in membrane structure/function, but we will consider strong applicants in all areas of biochemistry. Appointment at the Assistant Professor level requires a doctoral degree, postdoctoral experience, and evidence of outstanding research potential and the ability to develop a vigorous, independently-funded research program. In exceptional cases, appointment at more senior levels may be considered, and would require strong evidence of outstanding research accomplishments, including extramural funding and international recognition. Appointees will be expected to share in the Department's responsibilities for teaching undergraduate and graduate courses in biochemistry.

Bioinformatics –Rank Open

The Department of Microbiology in conjunction with the Institute for Genomic Biology solicits applications for an open rank, full-time tenure-track position in bioinformatics and computational biology. This position requires a doctoral degree, postdoctoral experience, and evidence of outstanding research potential. The individual sought for this position should be able to develop a cutting-edge research program involving computational analysis of microbial genomes. Possible research areas include the development and application of new computational approaches for genome annotation, for the identification of functions for unknown proteins, for reconstructing metabolic pathways, and for building computational models for metabolic and regulatory networks. Candidates with expertise in generating global physiomic profiles by integrating data from genomic, proteomic, and metabolomic studies or in the areas of pharmacogenomics, toxicogenomics, or pharmacogenetics will also be considered. The successful candidate will be housed in the new Institute for Genomic Biology. When this state-of-the-art facility opens in Fall, 2006 it is expected to house approximately 400 researchers across a variety of biological disciplines, providing ample opportunities for collaboration. This position is part of a campus-wide initiative in bioinformatics involving multiple hires in the past year and active participation by the National Center for Supercomputing Applications. Appropriately qualified applicants may also seek affiliation with the Department of Computer Sciences.

Cell Biology – Rank Open

One or more positions are available in the Department of Cell and Developmental Biology (http://www.life.uiuc.edu/cdb/) for outstanding candidates whose research addresses fundamental questions of modern cell biology. Applications for full-time positions at the Assistant, Associate and Full Professor levels will be considered, and highly qualified scientists at these levels are encouraged to apply. Appointment at the Assistant Professor level requires a Ph.D. and/or M.D. degree, postdoctoral experience, and evidence of outstanding research potential. Appointees at this level will be expected to develop a vigorous, independently funded research program. Appointment at the higher levels requires evidence of outstanding research accomplishments. Applicants at all levels will be responsible for undergraduate, graduate or medical cell biology teaching.

Pharmacology – Assistant Professor

The Department of Pharmacology in the College of Medicine and the School of Molecular and Cellular Biology invite applications for a full-time tenure-track faculty position at the Assistant Professor level. A Ph.D. and/or M.D. degree and postdoctoral experience are required for appointment to this position. Applicants should be qualified to teach basic principles of pharmacology to second year medical students in the College of Medicine. Appointees will be expected to develop a vigorous, independently-funded research program that addresses contemporary questions in biochemistry, cell and molecular biology, or physiology. Applicants may also be considered for an appointment in Chemical Biology in the School of Chemical Sciences (http://www.scs.uiuc.edu/), if appropriate.

Applications should clearly indicate the position(s) applied for and should be submitted to: School of Molecular and Cellular Biology Search, University of Illinois at Urbana-Champaign, 393 Morrill Hall, 505 S. Goodwin Ave., Urbana, IL 61801. The application must include a curriculum vitae with a complete list of publications, a concise summary of past research accomplishments, and future research plans. Please arrange to have no fewer than three letters of recommendation sent to the same address.

Electronic submissions as pdf or Microsoft Word files are encouraged and should be sent to mcbsearch06@life.uiuc.edu. To ensure full consideration, applications must be received by January 3, 2006. Applicants may be interviewed before the closing date; however, no hiring decision will be made until after that date.

The University of Illinois at Urbana-Champaign is an Affirmative Action, Equal Opportunity Employer.

FELLOWSHIPS

UNCF•MERCK SCIENCE INITIATIVE

"A mind is a terrible thing to waste"

MERCK

UNDERGRADUATE SCIENCE RESEARCH SCHOLARSHIP AWARDS

- 15 Awards Annually
- Scholarships up to $25,000
- Two Summer Internships at a Merck Research Facility

An applicant must:

- Be a full-time student at any four-year college or university
- Have junior year academic status
- Major in a life or physical science (first professional degrees excluded)
- Have a minimum cumulative GPA of 3.3 (4.0 point scale)

GRADUATE SCIENCE RESEARCH DISSERTATION FELLOWSHIPS

- 12 Fellowships Annually
- Fellowship Stipends up to $42,000
- Department Grants of $10,000
- Support for 12-24 months

An applicant must:

- Be enrolled full-time in a Ph.D. or equivalent doctoral program in a biomedical life or physical science
- Be engaged in and within 1-3 years of completing dissertation research

POSTDOCTORAL SCIENCE RESEARCH FELLOWSHIPS

- 10 Fellowships Annually
- Fellowship Stipends up to $70,000
- Department Grants of $15,000
- Support for 12-24 months

An applicant must:

- Hold a Ph.D. or equivalent degree in a biomedical life or physical science
- Be appointed as a new or continuing postdoctoral fellow by the end of 2006 at an academic or non-academic research institution (private industrial laboratories are excluded)

Applicants must be African American (Black), U.S. citizens or permanent residents, and attending an institution in the U.S.A. Applications must be postmarked by December 15, 2005
For application forms and more information, please contact your department chairperson or Jerry L. Bryant, Ph.D., at the **United Negro College Fund**, 8260 Willow Oaks Corporate Drive, P.O. Box 10444, Fairfax, VA 22031-4511, by fax (703) 205-3574, by e-mail at *uncfmerck@uncf.org*. Apply online or download from our website at *www.uncf.org/merck/*

POSITIONS OPEN

UNIVERSITY of VIRGINIA

ASSISTANT PROFESSOR OF RESEARCH POSITIONS

The Laboratory of Dr. Richard Santen, at the University of Virginia, Department of Internal Medicine, Division of Endocrinology and Metabolism, is seeking to fill two Assistant Professor of Research Positions. These positions will conduct molecular biologic, cell culture, confocal microscopic, xenograft, radioimmunologic, and tumor biologic studies to examine the role of estrogen in the proliferation, apoptosis, and cell invasion and motility of breast cancer cells. Also, these positions will design hypothesis oriented studies to critically examine the process of breast tumor growth and cell death.

M.D and/or PhD required plus at least three years postdoctoral experience. Experience with training technicians a plus, as these positions will also be responsible for directing technicians to conduct studies examining the role of estrogens in breast cancer growth and treatment. Positions will be required to present findings at National and International meetings and publish work in high impact, peer reviewed journals, and so experience in writing research papers and abstracts is encouraged. The ideal candidate will have knowledge in standard methods of molecular biology, cell biology techniques, immunocytochemistry, animal experimentation, morphology, and all regular pathology techniques.

Positions are open until filled. Please send CV and statement of Research Interests to: **Dr. Richard Santen, University of Virginia, PO Box 801416, Charlottesville, VA, 22908 or e-mail to: RJS5Y@virginia.edu**

The University of Virginia is an Equal Opportunity/ Affirmative Action Employer.

CSH COLD SPRING HARBOR LABORATORY

ASSISTANT PROFESSOR POSITIONS

CANCER BIOLOGY & STRUCTURAL BIOLOGY

Cold Spring Harbor Laboratory is seeking outstanding candidates to fill several faculty positions at the **Assistant Professor** level. Candidates must have completed at least 2 years of postdoctoral training and developed an innovative research program.

Several positions are available in **Cancer Biology.** CSHL is an NCI-designated Cancer Center, with research programs ranging from basic studies in gene expression and signal transduction pathways to animal models for cancer and cancer genomics.

One position is available in **Structural Biology** in the field of Macromolecular Crystallography in an area that complements the CSHL programs in cancer biology or neuroscience. CSHL is a member of the Participating Research Team (PRT) for beamline X26C at the nearby National Synchrotron Light Source at Brookhaven National Laboratory.

Applicants should submit their curriculum vitae, a brief description of research interests and career goals, and arrange for 3 letters of reference to be sent to **Facultyjobs@cshl.edu**. Additional information about CSHL can be obtained at **www.cshl.edu.** We are an equal opportunity employer.

ARIZONA STATE UNIVERSITY

FACULTY POSITIONS IN BIOMEDICAL INFORMATICS

Arizona State University is establishing a new Department of Biomedical Informatics. The Department has several faculty positions open at all levels.

Associate and Full Professor Positions

Applications and nominations are invited for ASSOCIATE and FULL PROFESSOR positions in the areas of bioinformatics (with a focus on human genome or cancer research), clinical informatics (with a focus on nursing, imaging, or hospital informatics), or public health informatics. The candidate must have earned a doctoral-level degree (e.g., Ph.D., M.D., D Sc.) in Biomedical Informatics, Biological Sciences, Computer Science, Medicine, or a closely related field by the appointment date. Application must show substantial evidence of research/scholarly activity, teaching and service in biomedical informatics or medicine appropriate to the rank being applied. Evidence of scientific, academic and organization leadership, educational innovation, and demonstrated effectiveness in establishing clinical partnerships are also desired. Selected candidates will participate in interdisciplinary research, teaching, training initiatives related to biomedical informatics; and to play an active role in developing the new Department of Biomedical Informatics. The Department of Biomedical Informatics anticipates enrolling its first students in the fall of 2006.

Assistant Professor Tenure-Track Positions

Applications are invited for tenure-track positions in the areas of bioinformatics (with a focus on human genome or cancer research), clinical informatics (with a focus on nursing, imaging, or hospital informatics), or public health informatics. The candidate must have earned a doctoral-level degree (e.g., Ph.D., M.D., D Sc.) in Biomedical Informatics, Biological Sciences, Computer Science, Medicine, or a closely related field by the appointment date. Applicant must show exceptional promise of establishing a vigorous, extramurally funded research program, and participate in interdisciplinary research, teaching, training initiatives related to biomedical informatics. Senior assistant professors with research and teaching portfolios are also encouraged to apply.

Application packages must include a cover letter, detailed curriculum vitae, research and teaching statements, copies of four of the most important publications, and the names, street addresses, and phone numbers of four references. Applicants are encouraged to submit their materials by e-mail (as attachments in either MS Word .doc or PDF format) to **bmi.recruiting@asu.edu**. Alternatively, the application package must be sent to: **Chair, Biomedical Informatics Search Committee, Arizona State University, and P. O. Box 878809, AZ 85287-8809**. Inquiries should be sent by e-mail to **bmi@asu.edu**. The closing date for receipt of applications is **January15, 2006**; if not filled, applications will be accepted weekly thereafter until the search is closed. Anticipated start date is August 16, 2006. Pending budget approval.

About the Department

The Department of Biomedical Informatics will be primarily located in downtown Phoenix, adjacent to the Phoenix Bioscience campus that will house the new Phoenix track of the University Of Arizona College Of Medicine, the Arizona State University College of Nursing, and various other allied health departments. The Department will be located in a brand new building, which will provide state-of-the-art research facilities and plenty of room for growth. The Department will serve as an integral part of Phoenix Bioscience Campus and will play a role in research and graduate education at the medical school and nursing school. This is in line with our President's vision about the New American University, where research and teaching occur in a transdisciplinary setting that rewards entrepreneurship, community engagement, and intellectual fusion. Joint or affiliate appointments with other entities on the Phoenix Bioscience Campus are possible.

The Department of Biomedical Informatics has strong connections to the local and regional clinical and biomedical research facilities. These include the Mayo Clinic in Scottsdale and the Mayo Clinic and Mayo Medical School in Rochester, Minnesota; the Barrow Neurological Institute, a leader in research and medical care related to the neurosciences and in neurological disorders; and Banner Health, which is one of the largest, nonprofit health care systems in the country. Researchers in the Department of Biomedical Informatics also work closely with the local genomics research community that includes the Translational Genomics Research Institute (TGen) and the International Genomics Consortium. Joint or affiliate clinical appointments with ASU's partners are possible.

The Department of Biomedical Informatics has close ties to the leading research and academic departments at Arizona State University. The Biodesign Institute, which represents one of Arizona's largest investments in the biosciences, is a new multimillion dollar research enterprise focused on integrating the life sciences with computing and engineering to design biologically inspired solutions for improving human health, the environment, and national security. The Institute for Computer and Information Science and Engineering (InCISE) is a collaboration of interdisciplinary research units that share expertise in Computer and Information Science. The Decision Theatre is an immersive modeling and visualization facility that has applications to biomedical data. The Department also has strong partnerships with the Department Computer Science, Department of Bioengineering, Department of Mathematics and Statistics, School of Life Sciences, and College of Law at Arizona State University. Joint or affiliate appointments with these Departments and Colleges are possible.

Additional information about the Department can be found at **http://cse.asu.edu/bmi.html**.

Arizona State University is an Equal Opportunity/Affirmative Action Employer. A Background check is required for employment.

SENIOR RESEARCH SCIENTIST
U. S. ARMY RESEARCH OFFICE
RESEARCH TRIANGLE PARK, NC

The Senior Level position is located in the U.S. Army Research Office which conducts broad theoretical and experimental programs of basic and applied research in physics, materials science, electronics, mathematical sciences, chemical and biological sciences, mechanics, environmental science, and communications and information research. The incumbent's principal area of responsibility will span two or more of the following activities: biotechnology, nanotechnology, microelectronics, solid-state physics, fluid mechanics, laser and optical sciences, atmospheric science, energetic materials, quantum theory, advanced materials/materials processing. The incumbent will be instrumental in developing significant technology transfer activities from basic research to applied research and in some instances through deployment into fielded Army systems. Leadership in interfacing the Army's technical needs with the extramural university community will be an important component of this position. The incumbent interacts with international engineers and scientists from industry and academia, and with scientists and engineers in Army laboratories. The incumbent uses these contacts and inherent expertise to assess priorities in the frontier areas of science and engineer relevant to the Army, and to implement technology transfers to the Army, and other military services and industry. It is anticipated that the individual research for this position would be performed at a local (Research Triangle area of North Carolina) university utilizing the Army Research Office staff research program.

Salary range is $107,550 - $140,300 depending upon individual qualifications and salary history.

For further information related to the position, contact: **Dr. David Mann, ATTN: AMSRD-ARL-RO, P.O. Box 12211, Research Triangle Park, NC, 27709-2211, (919) 549-4249, email: david.mann1@us.army.mil.**

You may obtain an application package and information from **www.usajobs.opm.gov**, (click on **"job openings"**, then **Vacancy Announcement DA-43-05**) Direct any procedural questions to: **HRMD, 2531 Crystal Drive, Taylor Bldg. 8th Floor, Arlington/Crystal City, VA 22202, ATTN: Tom Peters, (703) 602-2715.**

PENNSTATE

FACULTY POSITION IN RNA VIROLOGY
The Pennsylvania State University

The Department of Biology at Penn State University invites applications for a tenure track faculty appointment (open rank). We seek an outstanding scientist in RNA virology, whose research is internationally recognized and complementary to existing areas of expertise in our Department. The successful candidate will focus on molecular mechanisms underlying viral establishment, dynamics and pathogenicity in individual hosts with a perspective on population level ecology and evolution. For further information about the Biology department, see **http://www.bio.psu.edu**.

Please submit letter of intent along with a curriculum vitae, statements of research plans and teaching philosophy, copies of relevant publications and arrange for three letters of recommendation to be sent on your behalf to: **Chair of RNA Virology Search Committee, 208 Mueller Laboratory, Box W, Penn State University, University Park, PA 16802**. Review of applications will begin **December 15, 2005**.

Penn State is committed to Affirmative Action, Equal Opportunity, and the diversity of its work force. AA/EOE.

Berkeley
UNIVERSITY OF CALIFORNIA

FACULTY POSITION IN BIOENGINEERING

The Department of Bioengineering in the College of Engineering at the University of California, Berkeley invites applications for a tenure-track position at the assistant, associate, or full professor level in the area of quantitative biomedical science and bioinstrumentation. We especially welcome candidates who work at the molecular, cellular and tissue level. The anticipated start date for the position is July 1, 2006.

The Department of Bioengineering has a strong undergraduate degree program and a joint graduate program with the University of California, San Francisco (UCSF). The interdisciplinary bioengineering program at UC Berkeley offers outstanding opportunities for collaboration with distinguished researchers in related departments and colleges, as well at UCSF, Lawrence Berkeley National Laboratory, and within the greater Bay Area biotech community. This exceptional environment for teaching and research in a rapidly growing field will provide the successful candidate with a unique opportunity to provide intellectual and technological leadership in bioengineering.

We seek an individual with demonstrated excellence in the field to establish an active and innovative research program. The candidate will also teach in appropriate areas of science and engineering and should have a strong commitment to and potential for excellence in teaching and leadership. To learn more about our department please visit http://bioeng.berkeley.edu. Applicants should have (or be about to receive) a doctoral degree or equivalent in a relevant area of engineering or the physical or biological sciences.

Applicants should submit a curriculum vitae with a complete list of publications; a brief description of research accomplishments; a selection of publication reprints (five or less); and a brief statement of future research plans and teaching interests. Applicants should also arrange to have three letters of reference sent to the department directly. Potential reviewers are referred to the Statement of Confidentiality at **http://apo.chance.berkeley.edu/evalltr.html**. We prefer to receive application materials via email to the following address: **search921@berkeley.edu**. We will also accept hard copy applications and reference letters sent to:

Chair Dorian Liepmann, Department of Bioengineering,
459 Evans Hall MC 1762, University of California, Berkeley, CA 94720-1762.

The review of applications will commence on November 15, 2005; applications must be received by January 31, 2006 for consideration in this year's recruitment cycle.

The University of California is an equal opportunity affirmative action employer, committed to excellence through diversity.

COLUMBIA UNIVERSITY

School of Dental and Oral Surgery

Interdisciplinary Research Opportunities
Research Faculty Position(s)
Postdoctoral Research Fellowship(s)
Stem Cell Biology/Tissue Engineering/Regenerative Medicine

Multiple full-time research faculty and postdoctoral research fellowship positions are available at Columbia University Medical Center campus. This research involves a collaboration among the School of Dental and Oral Surgery (SDOS), the College of Physicians and Surgeons, and the Department of Biomedical Engineering at the School of Engineering and Applied Sciences. The primary appointment will be at SDOS. The central focus is to engineer human tissue and/or organ analogs under the sponsorship of multiple NIH grants. Good verbal and written communication skills required. Positions available January 1, 2006.

Research Faculty Position(s)

Minimum of 6–8 years' research experience in cell and molecular biology, stem cell biology, tissue engineering, polymer chemistry, and/or biomaterials. Successful candidate(s) will be expected to maintain an active research program and to obtain external funding. Although these positions are not currently on the tenure track, there is the opportunity for tenure track appointments in the future. Ph.D. in biology or engineering, or equivalent degree required. Animal surgery techniques preferred. Rank and salary commensurate with experience.

Postdoctoral Research Fellow(s)

Research interest in cell and molecular biology, stem cell biology, tissue engineering, polymer chemistry, and/or biomaterials required. Recent recipient(s) of the doctorate, or its professional equivalent, are encouraged to apply. Individuals with related experience who have training awards that allow retraining in a new discipline or specialty will also be carefully considered.

To apply, send via e mail a statement of career goals, specific research interest, and curriculum vitae to **Kathleen Flaudrick (kf2184@columbia.edu).**

Columbia University is an affirmative action/equal opportunity employer.

Group Leader Position – Growth Control

The Friedrich Miescher Institute invites applications for a tenure track group leader position in the Growth Control Programme. We are seeking an outstanding individual who will establish a vigorous and ambitious research programme aimed at fundamental questions in Cancer Biology. We are particularly interested in individuals who focus on signalling pathways and metastasis.

The Institute provides excellent core facilities for genomics, protein chemistry and proteomics, monoclonal antibody production, fluorescence-activated cell sorting, fluorescence imaging, histology and mouse genetics. A highly competitive start-up package will be provided. The Friedrich Miescher Institute, part of the Novartis Research Foundation, is an international biomedical research centre with 280 members, including 180 post-doctoral fellows and graduate students (for further information see www.fmi.ch).

The Friedrich Miescher Institute is situated in Basel, Switzerland, a city offering an outstanding scientific and cultural environment in the centre of Europe.

Formal applications, including a CV, names and contact details of three referees and a concise description of research interests and future plans should be addressed to:

Professor Susan Gasser, Director
Friedrich Miescher Institute
Maulbeerstrasse 66
4058 Basel, Switzerland

The closing date for applications is:
December 1st, 2005

UNIVERSITY OF CALIFORNIA, BERKELEY ECOLOGIST

The Department of Integrative Biology, University of California, Berkeley invites applications to a faculty position in Ecology at the Assistant Professor level. We are searching broadly, without regard to taxon or system, for individuals who integrate experimental field studies with theory. We will consider exceptional ecologists in all areas, but are particularly interested in those working on species interactions, biological invasions, the community-ecosystem interface, and other areas that complement current faculty strengths on campus. UCB provides outstanding access to field sites, including the UC Natural Reserve System.

Applicants must have a Ph.D., productive postdoctoral experience, and a demonstrated record of research excellence. Candidates must be strongly committed to developing an externally funded, internationally recognized, research program, and contributing significantly to both the undergraduate and graduate curricula through teaching and mentorship.

Submit a CV, statements of research and teaching interests, and the names and addresses of three references to: **Chair, Ecology Search Committee, Dept. of Integrative Biology, 3060 Valley Life Sciences Bldg. #3140, University of California, Berkeley, CA 94720-3140 USA.** The deadline for receipt of applications is **December 16, 2005.**

The University of California is an Equal Opportunity Employer committed to excellence through diversity.

Project 2061 AAAS

POSTDOCTORAL POSITIONS

Project 2061 is seeking to fill three positions either at the Postdoctoral Fellow or Research Associate level. Successful candidates will assist in developing resources to advance students' understanding of the fundamental science ideas needed to be science literate as described in various state and national content standards documents. The work involves elaborating and clarifying the content standards, identifying phenomena and representations that support student understanding of the ideas in the content standards, writing and field testing assessment items that test student understanding of those ideas, and reviewing and writing summaries of related research on student learning. This work is related to the Project's long-term mission of reform in K-12 science education.

Applicants should have completed a doctoral degree in a science discipline or in science education. They should have a demonstrated interest in the teaching and learning of science, and a willingness to analyze and apply fundamental science ideas not only within but also outside their area of expertise. The work is intellectually challenging and requires strong analytical, organizational, and writing skills and the ability to work well in a team environment. Three to five years prior teaching experience at the K-12 level is desirable but not required.

These positions offer the opportunity for individuals with a deep understanding of basic science ideas and the ability and interest in communicating those ideas to work at the national level to improve the science literacy of all. A statement of the ideals that guide the work of Project 2061 can be found in its seminal publication, Science for All Americans, on-line at **www.project2061.aaas.org**.

Address all inquiries along with curriculum vitae, grade transcripts, and three letters of recommendation to: **AAAS, Human Resources Department, 1200 New York Ave., NW, Suite #102, Washington, DC 20005**. The positions offered are for a one-year period to be renewed contingent on grant funding. You may also reach us by Fax at **202-682-1630** and e-mail at **hrtemp@aaas.org**. Visit us at **www.aaas.org**. Application materials should be received by **December 31, 2005**.

EOE. Non-smoking work environment.

WANT TO PLUG THE POWER OF SCIENCE INTO PUBLIC POLICY?

Become a AAAS Science & Technology Policy Fellow.

What better way to connect your scientific expertise to serve society? Yearlong fellowships offer career-advancing opportunities to work with Congress or a federal agency in one of six thematic areas: Congressional • Diplomacy • National & Global Security • Health, Education, & Human Services • Energy, Environment, & Natural Resources • Revelle Global Stewardship. Stipends begin at $64,000.

Work in Dynamic Washington D.C.
What better place to apply your expertise to the decision-making process that affects people everywhere? You can focus your experience on your interests in national or international issues while you add valuable new perspective to your scientific training.

Join a Network of 1,700 Fellows.
Applicants must have a PhD or equivalent doctoral-level degree from any physical, biological, medical, or social science, or any engineering discipline. Individuals with a master's degree in engineering and at least three years of post-degree professional experience also may apply. U.S. citizenship is required and federal employees are not eligible. Since 1973, AAAS Fellows have benefited from a growing and diverse network of colleagues.

Apply for 2006–07 Fellowships by 10 January 2006.
Fellowships are awarded in the spring and begin 1 September with a two-week orientation. To apply, contact AAAS:
Phone 202-326-6700
E-mail fellowships@aaas.org
Website www.fellowships.aaas.org

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Enhancing Public Policy, Advancing Science Careers
www.fellowships.aaas.org

AAAS
ADVANCING SCIENCE, SERVING SOCIETY

Max-Planck-Gesellschaft
Max Planck Society

Selbstständige Nachwuchsgruppen

Independent Junior Research Groups

The Max Planck Society invites applications from outstanding young scientists in all fields of research pursued by the Max Planck Society (Biology and Medicine; Chemistry, Physics and Technology; Human Sciences).

Successful applicants will have demonstrated the ability to perform excellent research. They will be offered an **Independent Junior Research Group Leader** position (W2; equivalent to associate professor level without tenure) including a five-year grant (research positions, budget, investments) at a **Max Planck Institute of their choice.**

In addition, Independent Junior Research Group Leader positions are available at the

Max Planck Institute for Medical Research, Heidelberg (2 positions)
and the
Max Planck Institute for Biological Cybernetics, Tübingen (1 position)

Applications should include a CV, a list of publications, copies of three publications, a one-page summary of scientific achievements, and a two-page research plan. For further information and detailed application instructions see

http://www.snwg.mpg.de

The Max Planck Society is committed to equal opportunities and to employing disabled persons.

The deadline for application is **December 20, 2005.**

Computational Chemistry and Biology Opportunities at D. E. Shaw Research and Development

Extraordinarily gifted computational chemists, biologists, and other computational scientists are sought to join a rapidly growing New York-based research group that is pursuing an ambitious, long-term strategy aimed at fundamentally transforming the process of drug discovery.

Candidates should have world-class credentials in computational chemistry, biology, or physics, or in a relevant area of computer science or applied mathematics, and must have unusually strong research skills. Relevant areas of experience might include protein structure prediction, the computation of protein-ligand binding affinities, the study of biologically important systems using molecular dynamics and/or Monte Carlo simulation, and the application of statistical mechanics to biomolecular systems—but specific knowledge of any of these areas is less critical than exceptional intellectual ability and a demonstrated track record of achievement. Current areas of interest within the group include molecular dynamics simulation of functionally significant globular and membrane proteins, the prediction of protein structures and binding free energies, structure- and ligand-based drug design, characterization of protein-protein, protein-nucleic acid and protein-lipid interactions, and the development of algorithms for biomolecular simulations.

This research effort is being financed by the D. E. Shaw group, an investment and technology development firm with approximately $16 billion in aggregate capital. The project was initiated by the firm's founder, Dr. David E. Shaw, and operates under his direct scientific leadership.

We are eager to add both senior- and junior-level members to our world-class team, and are prepared to offer above-market compensation to candidates of truly exceptional ability. Please send your CV (including list of publications, thesis topic, and advisor, if applicable) to sciencemag-cc@desrad.deshaw.com.

D. E. Shaw Research and Development, L.L.C. does not discriminate in employment matters on the basis of race, color, religion, gender, national origin, age, military service eligibility, veteran status, sexual orientation, marital status, disability, or any other protected class.

D E Shaw & Co

University of Massachusetts Boston

Dean, College of Science and Mathematics

The University of Massachusetts Boston invites applications and nominations for the position of Dean of the College of Science and Mathematics. The college has over 2,500 students pursuing graduate and undergraduate degrees. It consists of ten departments and programs; including those of Biology; Biochemistry; Chemistry; Computer Science; Engineering; Environmental, Earth, and Ocean Sciences; Mathematics; and Physics. Through these programs, the college offers ten baccalaureate degrees, five master's degrees, and doctoral degrees in Computer Science, in Environmental Biology, in Environmental, Coastal and Oceanic Sciences, in Green Chemistry, and in Molecular, Cellular and Organismal Biology.

UMass Boston is a Carnegie classified Intensive Research University located in one of the most diverse and intellectually rich cities of the United States. Its undergraduate and graduate enrollment of about 12,000 students makes it the second largest campus of the University of Massachusetts system. Its six colleges are composed of over 800 full-time and part-time faculty, who provide over 150 academic programs leading to baccalaureate, masters, and doctoral degrees (www.umb.edu).

Applicants for the position must possess: an earned doctorate in an appropriate discipline; an outstanding record of teaching, scholarship, and service worthy of being granted tenure in an academic department; excellent leadership and communication skills; and commitment to academic excellence, diversity, and service. Applicants must also demonstrate a commitment to interdisciplinary and intercollegiate collaboration and an ability to build supportive ties and strong research programs within the college and with external stakeholders within local, national, and international communities.

The dean is the college's chief administrative and academic officer and reports directly to the provost and senior vice chancellor for academic affairs. The dean has responsibility for all aspects of curriculum planning and development, faculty and staff hiring, development and evaluation, and budget. He/she will be expected to help marshal the vibrancy and creative energies of the faculty, provide visionary leadership, and offer specific strategic steps necessary to lead the college to national prominence. The dean will be joining a new chancellor and provost and will have the opportunity to make new strategic hires to help mold an exciting future for the university. The salary for the position is competitive, commensurate with experience and qualifications.

Applications and nominations will be reviewed on an ongoing basis beginning the end of October 2005 and evaluated until the position is filled. Applicants must submit a cover letter addressing the qualifications delineated above, a curriculum vitae, and the names, addresses and telephone numbers of four references to: **Office of the Provost and Senior Vice Chancellor for Academic Affairs, UMass Boston, 100 Morrissey Blvd., Boston, MA 02125-3393, Attention: Dean Search.**

UMass Boston is an Affirmative Action, Equal Opportunity, Title IX employer and strongly encourages women, members of all ethnic groups, and people with disabilities to apply.

University of Massachusetts Boston
UMASS www.umb.edu

THE STATE UNIVERSITY OF NEW JERSEY
RUTGERS

Tenure Track Faculty Positions Computational Biology, Molecular Biophysics, and Systems Biology

The BioMaPS Institute for Quantitative Biology at Rutgers University invites applications for tenure track faculty positions at the junior or senior level in computational biology, molecular biophysics, and systems biology. The positions will be joint with an affiliated department in the Faculty of Arts and Sciences or in Engineering. Areas of interest include but are not limited to: the structure and function of molecular and cellular machines, biological networks, structural genomics and proteomics. Applicants should submit a cover letter, curriculum vitae, research summary and statement of future research goals, and a statement of teaching experience and interests and arrange for four letters of recommendation to be sent on their behalf. Materials should be submitted electronically as PDF files to: **Dr. Paul Ehrlich, Administrative Director, BioMaPS Institute for Quantitative Biology, Rutgers University, Piscataway NJ 08854** (email: **pehrlich@biomaps.rutgers.edu**). Currently BioMaPS Institute faculty hold joint appointments with the Departments of Chemistry, Mathematics, and Physics in the Faculty of Arts and Sciences at Rutgers University, New Brunswick Campus. For more information about the BioMaPS Institute, the applicant is directed to: **http://www.biomaps.rutgers.edu**. The review of applications will begin on **December 1, 2005**. *Rutgers University is an Affirmative Action/Equal Opportunity Employer. Women and minority candidates are especially encouraged to apply.*

POSITIONS OPEN

PHYSICIST
JILA, University of Colorado and National Institute of Standards and Technology, Boulder, Colorado

JILA, a premier academic research institute administered jointly by National Institute of Standards and Technology (NIST), and the University of Colorado, is searching for outstanding Scientists preferably at the junior level. Successful applicants would be expected to establish an internationally-recognized research program involving graduate, undergraduate, and postdoctoral students, and to participate in departmental teaching responsibilities. We have particular interest in candidates applying advanced techniques to topics related to JILA's strengths in atomic, molecular and optical science, laser technology, and precision measurement. Target areas include, but are not limited to, quantum information, quantum optics, quantum control, high-field physics, chemical physics, nanoscience, and biophotonics. JILA has a number of exceptionally successful faculty from underrepresented groups, and especially seeks applications from women and minority researchers.

More information about JILA can be found at **website: http://jilawww.colorado.edu.**

Interested persons should send curriculum vitae and a detailed research proposal (two to three pages), as well as arrange for three letters of recommendation to be sent to: **Physics Search Committee, JILA 440 UCB, University of Colorado Boulder, CO 80309-0440.**

Application review will begin December 15, 2005, and will continue until January 5, 2006.

For further information, contact: **Deborah Jin, e mail: jin@jilau1.colorado.edu, telephone: 303 492-0256,** or **Pam Leland, e-mail: leland@jila.colorado.edu, telephone: 303 492 4763.** *The University of Colorado, Boulder and NIST are both committed to diversity and equality in education and employment.*

POSITIONS OPEN

OHIO UNIVERSITY 1804

FACULTY POSITION NEUROMUSCULAR BIOLOGY

The Department of Biomedical Sciences of the Ohio University College of Osteopathic Medicine (**website: http://www.oucom.ohiou.edu/dbms/index.htm**) seeks applicants for a tenure-track faculty position at the **ASSISTANT** or **ASSOCIATE PROFESSOR** level. Responsibilities for the successful applicant are: (1) to develop an independent, externally funded research program in the context of the Interdisciplinary Institute for Neuromusculoskeletal Research (**website: http://www.oucom.ohiou.edu/IINR**), and (2) to participate in a medical physiology curriculum emphasizing student engagement and active learning. It is hoped that the candidate will grow into a leadership role within the Institute, which encompasses basic scientists, clinicians and engineers. The Institute is funded through 2010 by an extramural grant supporting research infrastructure. A Ph.D. (or equivalent) is required and postdoctoral training is desirable. The appointment begins as early as July 1, 2006. The 11 month salary will be commensurate with experience and accompanied by an excellent benefits package. Review of applications will begin December 15, 2005, but new applications will be reviewed until the position is filled. Submit a statement of teaching and research interests, curriculum vitae, representative reprints, and the names of three references to: **John N. Howell, Ph.D., Search Committee Chair, Department of Biomedical Sciences, Ohio University College of Osteopathic Medicine, 228 Irvine Hall, Athens, OH 45701. E mail: howell@ohio.edu.** *Ohio University is an Affirmative Action, Equal Opportunity Employer with a dual career network (website: http://www.ohio.edu/dual).*

POSITIONS OPEN

GEOGRAPHIC INFORMATION SYSTEM/ SPATIAL INTEGRATIVE BIOLOGIST

St. Louis University, a Catholic Jesuit institution dedicated to student learning, research, health care, and service is seeking applicants for a tenure track Assistant Professor position in the Department of Biology. Research interests in Spatial Integrative Biology required. We seek an individual with expertise in any area of biology whose research involves the spatial distribution of organisms or processes, understanding how these are achieved and maintained, and/or the integration of patterns and processes at multiple scales. Expertise in geographical information systems (GIS) is essential and expertise in a particular group of organisms, ecology, population genetics, evolution, or biogeography preferred. The successful candidate will be expected to develop an independent, extramurally funded research program and to contribute to our undergraduate and graduate curricula. Excellent facilities and a competitive start up package are provided, and opportunities are available to collaborate with researchers at nearby Universities, Missouri Botanical Garden, Danforth Plant Science Center, Missouri Department of Conservation, Army Corps of Engineers, and St. Louis Zoo. Applicants should have a Ph.D., postdoctoral experience, and a record of research productivity. All applications must be made online at **website: http://jobs.slu.edu;** applications must include curriculum vitae and separate statements of teaching and research goals. Send reprints and three letters of recommendation by post to: **Dr. Richard L. Mayden, Department of Biology, St. Louis University, 3507 Laclede Avenue, St. Louis, MO 63103-2010.** Information about the Department and position is at **website: http://bio.slu.edu.** Review of applications will begin November 28, 2005, and will continue until suitable candidates are identified. *St. Louis University is an Affirmative Action, Equal Opportunity Employer, and encourages nominations and applications of women and minorities.*

School of Medicine
Department of Community and Environmental Medicine
UNIVERSITY OF CALIFORNIA IRVINE

The department is seeking to fill a position for an Assistant (tenure-track) or Associate Professor or Professor (tenured) in the School of Medicine. Demonstrated success in publication in flagship journals in the area of toxicology, environmental health sciences and/or public health research is required. Evidence of peer-reviewed funding is required in a research area related to the adverse effects of environmental chemicals on human health, with strong emphasis at the molecular level, or related areas in environmental health sciences. A record of effective teaching skills is essential, as the successful candidate will be expected to contribute significantly to graduate teaching in environmental toxicology and public health. Applicants must possess the Ph. D. or M. D. degree, and postdoctoral training in toxicology or environmental health sciences is preferred. Salary will be commensurate with experience.

Send curriculum vitae and names of three references to: **Professor Ronald C. Shank, Department of Community and Environmental Medicine, University of California at Irvine, Irvine, CA 92697-1825.** Closing date is **January 6, 2006**.

The University of California, Irvine has an active career partner program and an NSF ADVANCE program for Gender Equity and is an Equal Opportunity Employer committed to excellence through diversity.

UNIVERSITY OF NORTH CAROLINA WILMINGTON

The Business of Marine Biotechnology Postdoctoral Fellowships

The Center for Marine Science at the University of North Carolina Wilmington is offering two exceptional postdoctoral fellowships in marine biotechnology. Candidates must have a PhD in a biotechnology-related area and are expected to conduct research in marine science laboratories at the University while pursuing a professional MBA degree in the University's Cameron School of Business. The goal of this 24 month program is to produce individuals with a solid science background as well as the business skills needed to prosper in a modern competitive business environment. Students in the MBA portion of the program will master the core functions of business, develop analytical and quantitative business skills, and study current and future business issues through real world experiences with regional companies involved in marine biotechnology. The research portion of the program involves working in one of 3 focus areas: 1. Bioassay technique development focusing on novel sensing methods with particular application in the marine environment; 2. Finfish mariculture which may include genetics and selective breeding, recirculating aquaculture technology, nutrition, and commercial demonstration; and 3. Marine pharmaceuticals and nutraceuticals from cultured organisms, bioengineered natural products, novel enzymes and biosynthetic pathways. Candidates should clearly identify their interests in one of the 3 focus areas in their cover letter. Selected candidates would receive salary and benefits including health insurance and retirement contributions for 24 months. Position title will be "Visiting Research Assistant Professor". Tuition for the coursework necessary to obtain the MBA is also provided.

Screening of the applicants will begin **January 15, 2006** and applicant would be required to start May 1, 2006 and all degree requirements must be met at that time to qualify for the fellowship. Letter of application, curriculum vitae, summary of research plans, and names and addresses of three references should be sent via the online application process available on the Web at **http://consensus.uncw.edu** not emailed or mailed. MS Word or Adobe PDF attachments are strongly preferred. For questions regarding the online applications process, contact **Kathleen Ludeman** at **910-962-2493**. For questions regarding the positions or the Center contact **Dr. Ronald K. Sizemore** at **Sizemorer@uncw.edu** or visit our website at **http://www.uncw.edu/cmsr**.

UNCW is an Affirmative Action, Equal Opportunity Employer. Women and minorities are encouraged to apply.

Faculty Recruitment in Basic and Translational Research

New York University School of Medicine announces a major expansion of its Program in Cardiology Biology, under the direction of **Drs. Glenn I. Fishman and Edward A. Fisher**, at the new Joan and Joel Smilow Research Building. We are seeking up to five new tenure track faculty recruits engaged in basic and/or translational research related to cardiac and vascular biology and disease. All will be members of the Program in Cardiovascular Biology with primary academic appointments in one of the basic science or clinical departments. Laboratories will be available in the new Smilow Research Building scheduled for occupancy in February 2006. Positions are available at all levels, including Assistant, Associate or full Professor. Areas of interest include, but are not limited to: Electrophysiology, Myocyte Growth and Death, Vascular Biology and Disease, Cardiovascular Development, Animal Models of Cardiovascular Disease, and Stem Cells and Regenerative Medicine. Investigators whose research will enhance the translation of basic research findings into new therapeutic interventions and the design of new clinical trials are encouraged to apply. Successful applicants should hold an MD and/or PhD with established reputations in these areas of research and visibility at the national level.

Interested investigators are encouraged to visit **http://www.med.nyu.edu/smilowcenter/opportunities/cardiovascular_opps.html** to learn more about the NYU Program in Cardiovascular Biology, qualifications for candidates and our recruitment process. Qualified candidates can apply by following the instructions found on that web site. All application packets should be sent to **cvbsearch@med.nyu.edu**.

The Cardiovascular Biology recruitment efforts are part of a larger NYU School of Medicine recruitment initiative. For more information, please visit **http://www.med.nyu.edu/smilowcenter**.

DIRECTOR
University of Connecticut Stem Cell Institute

The University of Connecticut invites applications for the DIRECTOR of the University of Connecticut Stem Cell Institute (UCSCI).

UCSCI is a cross-campus initiative that will be staffed by existing scientists as well as new recruits, all of whom are expected to establish research programs of international distinction in the broad area of stem cell biology and regenerative medicine. With the enthusiastic support of stem cell research by the State of Connecticut, the Director will have a unique opportunity to establish a vibrant research enterprise with significant support.

The Director of UCSCI will enjoy an outstanding resource package containing generous start-up funds and space in a new state-of-the-art research building near the Farmington campus of the University of Connecticut School of Medicine. The Director will also spearhead the recruitment of new faculty members of the Institute with the option of tenure-track appointments at either the Health Center or Storrs campus of the University of Connecticut.

The ideal candidate should be the leader of an internationally recognized research program in areas such as embryonic, adult, hemopoietic or cancer stem cells and have the academic background and leadership qualities to ensure vigorous growth and enhance synergistic and thematic interactions within the Institute.

Applicants should submit a complete curriculum vitae, a statement of research interests and direction by **January 1, 2006**. Applications should be transmitted electronically in RTF or PDF format to **ucsci@uchc.edu**.

The University of Connecticut is an Equal Opportunity/ Affirmative Action Employer.

POSITIONS OPEN

FACULTY POSITION
Nutritional Metabolomics

The Departments of Food Science and Human Nutrition (website: **http://www.clemson.edu/foodscience/**) and Genetics and Biochemistry (website: **http://www.clemson.edu/genbiochem/**) at Clemson University invite applications for a tenure-track position in the area of human nutritional metabolomics at the level of Associate or Full Professor. Qualifications include a Ph.D. and a history of external research funding. The successful candidate will be expected to develop a competitive, extramurally funded research (75 percent) program of national distinction using bioinformatics tools, nutrigenomics knowledge and modern molecular techniques, and to supervise and mentor undergraduate and graduate research. Experience that includes a clinical component is desirable. Teaching (25 percent) undergraduate and graduate courses in the nutrition and metabolism areas is also anticipated.

The successful applicant will be a collaborator within the biomedical emphasis area and would be an interface with the Departments of Biological Sciences and Bioengineering and the Greenwood Genetics Center. This individual will also be expected to build strong collaborations with the South Carolina Nutrition Research Consortium (website: **http://www.scnrc.org/**) to enhance our ability to focus on the discovery of basic mechanisms that could be used in the development of strategies to prevent nutrition related diseases, and to build a nationally recognized integrated molecular nutrition graduate program.

This position offers competitive startup funds and an attractive salary. Laboratory space will be provided in the Department of Food Science and Human Nutrition with ample opportunities to access cutting edge research instrumentation for high throughput functional genomics and proteomics housed in the Genomics Institute and the Department of Genetics and Biochemistry.

To apply, submit a letter of application, curriculum vitae, statements of teaching and research interests, and the names and addresses of three potential references as a single PDF file to: **Dr. Felix Barron** at e mail: **fbarron@clemson.edu.** Please include "**Metabolomics**" in the subject heading. Applications not in a single PDF file will be returned and must be resubmitted in the correct format. Deadline for receipt of applications is February 7, 2006.

We especially encourage minorities and women to apply. Clemson University is committed to Affirmative Action, Equal Opportunity, and the diversity of its workforce. "Clemson University does not discriminate against any person or group on the basis of age, color, disability, gender, national origin, race, religion, sexual orientation, or veteran's status." An offer of employment is contingent upon establishment of identity and verification of employment eligibility as required by the Immigration Reform and Control Act of 1986.

Department of Wildlife and Fisheries Sciences
Faculty of Ecology and Evolutionary Biology
Genomics Signature Program at
Texas A&M University

We seek a tenure-track **ASSISTANT PROFESSOR** with research expertise in comparative genomics and/or quantitative genetics of vertebrates as applied to systematics, evolution, conservation, and management of natural populations. The candidate must establish an independently funded graduate research program, and teach in his/her area of expertise. Submit curriculum vitae, statement of research and teaching interests, relevant reprints, and have three letters of reference sent to: **Dr. John W. Bickham, Department of Wildlife and Fisheries Sciences, 210 Nagle Hall, Texas A&M University, College Station, Texas 77843-2258. Telephone: 979 845 5777. E mail: j bickham@tamu.edu.** Reviews of applications will begin January 1, 2006; start date is expected to be September 1, 2006.

The Texas A&M University System is an Equal Opportunity Employer and encourages applications from women and minorities.

POSITIONS OPEN

HARVARD UNIVERSITY
Department of Earth and Planetary Sciences

The Department of Earth and Planetary Sciences at Harvard University seeks to fill a faculty position at the **ASSISTANT** or **ASSOCIATE PROFESSOR** level (untenured) in the broadly defined area of Geobiology. The individual may bring strength to the Department in the area(s) of paleontology, microbial biology, evolutionary biology, Earth history, and/or geochemistry, but the search is not limited to these sub-disciplines. This new position is part of a broad initiative for growth in the Department of Earth and Planetary Sciences and may be coordinated with allied departments such as Organismic and Evolutionary Biology, the Division of Engineering and Applied Sciences, or with the new Microbial Sciences Initiative. Applicants should send (by mail or e mail) a statement of research and teaching interests, curriculum vitae, and the names and contact information, including e mail addresses, of three references to:

Geobiology Search Committee
c/o Jason Miller
Department of Earth and Planetary Sciences
Harvard University
20 Oxford Street
Cambridge, MA 02138 USA
E mail: miller@eps.harvard.edu

Applications will be reviewed beginning December 15, 2005. For more information about the Department, please visit our **website: http:// www.eps.harvard.edu.** *We particularly encourage applications from women and minorities. Harvard University is an Affirmative Action/Equal Opportunity Employer.*

EASTERN MICHIGAN UNIVERSITY
Cellular and Molecular Biology

The Department of Biology invites applications for a tenure-track position in cell and molecular biology at the Assistant Professor level, beginning August 2006. We seek an individual with a Ph.D., postdoctoral experience, and ability to teach at the college level. The position also requires evidence of research in cell and molecular biology. Preference may be given to candidates with expertise in bioinformatics. The successful candidate will be expected to teach core courses such as cell and molecular biology, and cell-molecular biology and genetics lab, as well as carry out an active research program.

Please send a letter of interest for Posting F0618 and include curriculum vitae, statement of teaching interests and philosophy, description of research, up to three current reprints, graduate transcripts (unofficial acceptable), and three letters of reference to: **Dr. Tammy Greco, Department of Biology, 316 Mark Jefferson, EMU, Ypsilanti, MI 48197.** Review of applications will begin December 2, 2005, and continue until the successful candidate is hired. For additional information, contact: **Dr. Greco, telephone: 734 487 4242. E mail: tgreco@emich.edu. Fax: 734- 487-9235. Website: http://www.emich.edu/biology/.** *Eastern Michigan University is an Affirmative Action/Equal Opportunity Employer. We encourage women and members of minority groups to consider this opportunity.*

CELL BIOLOGY
University of Puget Sound

Full time, tenure line Assistant Professor; begins fall term 2006. Ph.D. with emphasis in cell biology required. Postdoctoral teaching or research experience desirable. For complete job description and application procedures, visit **website: http:// www.ups.edu/employment.xml.** Application deadline is December 1, 2005. *The University of Puget Sound is an Equal Opportunity, Affirmative Action Educator/Employer.*

POSITIONS OPEN

The Department of Biological Sciences at the University of Nevada Las Vegas (UNLV) (**website: http://biology.unlv.edu/**) invites applications for a full time, nine month, tenure track **ASSISTANT, ASSOCIATE,** or **PROFESSOR** in microbiology, commencing fall 2006. The Department has active undergraduate and graduate programs (M.S. and Ph.D.) and a diverse faculty of 27 individuals representing strengths in physiology, ecology and evolutionary biology, cell and molecular biology, and microbiology. Research area is open, but preference may be given to an individual who addresses current problems in microbial ecology, pathogenesis, genetics and/or physiology using molecular biology or bioinformatics approaches. Minimum requirements include a Ph.D. in microbiology or closely related field from an accredited college or university, postdoctoral experience and a record of creative and significant research in microbiology. Currently, the Department has a growing core of faculty in microbiology (**website: http://www.unlv.edu/faculty/hjwing/microbiology.htm**) and it is expected that the successful candidate will interact with and support this core, develop a vigorous extramurally funded research program and contribute to teaching and mentoring in the Department's B.S., M.S. and Ph.D. programs. Excellent core research facilities are available through recent National Science Foundation Experimental Program to Stimulate Competitive Research and NIH IdeA Networks of Biomedical Research Excellence infrastructure awards. Salary is competitive; contingent on labor market and contingent upon funding. Please submit a cover letter, curriculum vitae, a list of three professional references with contact information, and statements of research and teaching interests/philosophy along with citations of three key publications to: **Dr. Eduardo Robleto, Search Committee Chair.** Materials are to be submitted via online application at **website: https://hrsearch.unlv.edu.** For assistance with UNLV's application system, please contact **Bob Sitts** at **telephone: 702-895-1655,** or **e-mail: hrsearch@unlv.edu.** (PN 247 SN 6185)

Review of all applications will begin December 15, 2005, and continue until the position is filled.

UNLV is an Affirmative Action/Equal Opportunity Education and Employer committed to excellence through diversity.

MANAGER OF NORTH AMERICAN FOOT AND MOUTH DISEASE VACCINE BANK

The U.S. Department of Agriculture, APHIS, Foreign Animal Disease Diagnostic Laboratory on Plum Island, New York, is seeking a full-time Veterinary Medical Officer (GS 12/13; annual salary of $63,103 to $97,553 plus benefits). Incumbent will serve as the Manager of the North American Foot-and-Mouth Disease Vaccine Bank (NAFMDVB). The NAFMDVB is a collaboration among Canada, Mexico, and the United States which purchases, evaluates, and stores vaccine antigen concentrates for strains of foot-and-mouth disease virus (FMDV).

Incumbent must have an in-depth knowledge and extensive experience in Foot and Mouth (FMS) vaccine development and manufacturing as well as a thorough understanding of international standards for evaluation of the efficacy and purity of FMD vaccines. In addition, incumbent should have a good understanding of serological and molecular biological techniques used to evaluate the status of animals exposed to FMDV, and a thorough knowledge of clinical aspects of FMD in livestock.

The incumbent must be a U.S. citizen and able to obtain a secret security clearance while employed for the position. A degree of Doctor of Veterinary Medicine with advanced training (Ph.D. preferred) in microbiology or a related discipline is required. The position is a two-year term appointment which is renewable. A recruiting bonus up to 25 percent of the annual salary may be offered. Deadline for application is November 7, 2005. A copy of announcement (job number: 2487 2005 0588) can be obatined at **website: http://jobsearch.usajobs.opm.gov** or **telephone: 631-323-3256/3206** for application procedures. *The Federal Government is an Equal Employment Opportunity Employer.*

University of Wisconsin Medical School
McArdle Laboratory for Cancer Research
Faculty Position in Cancer Research

We are looking for a colleague to join us in a tenure-track faculty position as an Assistant Professor in the McArdle Laboratory for Cancer Research **(http://mcardle.oncology.wisc.edu)** at the University of Wisconsin Medical School. The eighteen faculty at the McArdle Laboratory are committed to understanding the origins, prevention, and therapy of cancer through basic and translational research. Research areas of particular interest for this search include cancer genetics, cancer cell biology, and the development of targeted therapies for the prevention or treatment of cancer, but candidates in all areas of basic cancer research will be considered. Members of the department participate in the University of Wisconsin Comprehensive Cancer Center and in excellent, well-funded graduate and postdoctoral training programs. Faculty in the department have access to superb animal facilities, a genomics facility, a high-throughput small molecule screening facility, flow cytometry, experimental pathology, informatics, and biostatistics. Applicants are expected to have a Ph.D. degree and significant research accomplishments.

To be considered, please submit a curriculum vitae, publication list, a 2-3 page statement of research accomplishments and future objectives, and have 3 qualified individuals send letters of recommendation. Review of the applications will begin on **December 15, 2005**. Applications and letters of recommendation should be sent to: **Faculty Search Committee, McArdle Laboratory for Cancer Research, University of Wisconsin, 1400 University Avenue, Madison, WI 53706-1599**.

The University of Wisconsin is an Equal Opportunity/Affirmative Action Employer. Minority and women candidates and all other qualified persons are encouraged to apply. Under Wisconsin statutes, names, positions and addresses of applicants and nominees may be subject to release upon request.

PURDUE UNIVERSITY
Faculty Position in Computation

The Department of Medicinal Chemistry and Molecular Pharmacology at Purdue University invites applications for a tenure-track position. This position is part of an interdisciplinary vision to build on existing strengths at the chemistry/biomedical sciences interface **(www.mcmp.purdue.edu)**. Candidates should use computational methods to solve chemical, biological or biophysical problems and have current or future interests relevant to the broadly defined area of drug design including diagnostic, therapeutic, or preventive agents. Research could include (but is not limited to) computation to predict systems behavior, or molecular modeling on levels ranging from detailed atomic models to low-resolution models and multiscale methods. The research in the Department is an integral component of the interdisciplinary activities across the Purdue campus including the Purdue Cancer Center and the Centers of Discovery Park **(www.mcmp.purdue.edu/research.php)**.

Candidates must have a Ph.D. degree and post-doctoral experience and show exceptional promise in research and a commitment to excellence in teaching at the undergraduate and graduate levels. Experience with computation focused on molecular systems of biomedical relevance is preferred. Outstanding senior candidates with appropriate research interests and scholarly achievements will be considered for appointment to a tenured position. Submit curriculum vitae, a summary of planned and/or ongoing research, and three letters of reference to: **Computation Faculty Search, Purdue Univ, Department of Medicinal Chemistry and Molecular Pharmacology, 575 Stadium Mall Dr, W Lafayette, IN 47907-2091**. Applications will be reviewed beginning **December 1, 2005**.

Purdue Univ. is an Equal Opportunity/Equal Access/Affirmative Action Employer fully committed to achieving a diverse workforce. Women, minority applicants, and dual career couples are encouraged to apply.

Yale UNIVERSITY
Department of Molecular, Cellular and Developmental Biology

The Department of Molecular, Cellular and Developmental Biology of Yale University invites applications for either a junior or senior faculty appointment in **Computational Biology**. The Department is particularly interested in individuals with expertise in mathematics or computer science who combine theory and experiment to solve important problems in cellular, molecular or developmental biology, including neuroscience. The successful candidate is expected to lead an active research group and participate in interdisciplinary research and training. The successful candidate should also demonstrate excellence in teaching at both the undergraduate and graduate levels. Review of applicants will begin January 2, 2006 and the search will remain open until the position is filled.

Information on the Department can be found on our web site: **http://www.mcdb.yale.edu**.

Please submit curriculum vitae and description of research interests to: **"Search Committee" either by e-mail to aileen.donnelly@yale.edu or by mail to Department of Molecular, Cellular and Developmental Biology, Yale University, P.O. Box 208103, New Haven, CT 06520-8103.** Candidates for assistant professor should also request three letters of recommendation addressed to the Search Committee. *Yale University is an Affirmative Action/Equal Opportunity Employer. Women and underrepresented minority scholars are especially encouraged to apply.*

Incyte is a Wilmington, Delaware-based drug discovery and development company with active internal drug discovery programs focused on the identification of novel small molecule drugs for inflammation, cancer and diabetes. Under a collaborative licensing agreement, Incyte is developing Reverset™, a novel nucleoside analog reverse transcriptase inhibitor, which is in Phase II development, to treat human immunodeficiency virus (HIV) infections.

BIOCHEMIST

Incyte Corporation is looking for a broadly trained biochemist with experience in protein functional characterization, enzymology and/or protein chemistry. The successful candidate will interact broadly with biologists and chemists in support of our ongoing early stage drug discovery efforts. Experience with molecular or cell biology is a plus. A Ph.D. degree plus postdoctoral experience is required.

Incyte is uniquely located close to Philadelphia, NY City, Baltimore, D.C., New Jersey/Delaware beaches and Pocono Mountain resorts. We offer a competitive salary, 401K and other benefits. For consideration, please send your resume to careers@incyte.com, referencing Job Code LL6416RW. To learn more, please visit our website at **www.incyte.com**. Incyte Corporation is proud to be an Equal Opportunity Employer and recognizes the talent of its diverse workforce. EOE F/M/V

Incyte

POSITIONS OPEN

CHAIR, DIVISION OF PHARMACOLOGY
University of Missouri, Kansas City
School of Pharmacy

The School of Pharmacy is seeking a qualified and motivated individual for the position of Chair of the Division of Pharmacology. The responsibilities of this position include: administration of a team of eight faculty members responsible for pharmacology instruction to pharmacy, dental, graduate, and nursing students; mentoring and recruitment of new faculty; coordination of both division and interdisciplinary research programs; and an active individual research program. This position reports directly to the Dean of the School of Pharmacy and has interactions with other units involved in both professional and graduate education.

The successful candidate should have a terminal doctoral degree (Ph.D., Pharm.D., M.D.) and significant research accomplishments including a high level of extramural research funding which would qualify the individual for the rank of professor. Preference will be given to individuals with prior administrative experience and/or an exemplary record in professional leadership. Salary will be commensurate with experience. The position will be available on or after April 1, 2006. For full consideration, applications should be received by February 1, 2006.

University of Missouri, Kansas City (UMKC) is a comprehensive research university exemplifying the values of education first, innovation, accountability, diversity, and collaboration. The School of Pharmacy is one of the four health professional schools on the UMKC campus, and is engaged in a University wide initiative to advance the life sciences in the Kansas City corridor through the Kansas City Area Life Sciences Institute (KCALSI) **(website: http://www.kclifesciences.org)**. The Division of Pharmacology is one of three divisions in the School of Pharmacy, interfacing with the Divisions of Pharmaceutical Sciences and Pharmacy Practice. Construction of a new facility to house the School of Pharmacy is underway, and the successful candidate will have an opportunity for extensive input into the Pharmacology space. More about UMKC is at **website: http://www.umkc.edu/strategicplan**, or go to **website: http://www.umkc.edu/pharmacy**.

Nominations and applications indicating interest in the position, complete curriculum vitae, and three letters of reference should be forwarded to:

Robert W. Piepho, Ph.D.
Chair, Search Committee
School of Pharmacy
University of Missouri-Kansas City
5100 Rockhill Road
Kansas City, MO 64110 2499
Telephone: 816-235-1609
E mail: piephor@umkc.edu

UMKC is an Affirmative Action/Equal Opportunity Institution.

University of Illinois, Chicago, Department of Chemistry is entering a phase of long term growth and invites applications for a **POSITION IN ALL AREAS OF BIOCHEMISTRY**, including structural biology and chemical biology. Although preference will be given to tenure-track Assistant Professors, applications at all levels are welcome. The successful candidate will be expected to carry out a full and vigorous program of innovative research and to contribute to the teaching of graduate and undergraduate students. Please submit applications, including curriculum vitae, list of publications, summary of past research, plans for future research, and letters of reference from three individuals who are familiar with the candidate's work, by November 29, 2005, to: **Wonhwa Cho, Biochemistry Search Committee, Department of Chemistry (M/C 111), The University of Illinois at Chicago, 845 W. Taylor Street, Chicago, Illinois 60607 7061.** *UIC is an Affirmative Action/Equal Opportunity Employer. Women and Minority Candidates are strongly encouraged to apply.*

RESEARCH ASSISTANT PROFESSOR. Nontenure track position at the University of Illinois at Chicago, Department of Anesthesiology, for research in molecular biology and/or mitochondrial metabolism. Ability to work independently and establish needed procedures. Able to draft own publications from research studies. Salary commensurate with experience. For fullest consideration, submit applications by November 21, 2005, to: **Dr. June Palmer, University of Illinois at Chicago, Department of Anesthesiology, m/c 515, 1740 W. Taylor, Chicago, IL 60612.** *Affirmative Action/Equal Opportunity Employer.*

INTEGRATIVE PHYSIOLOGIST TENURE-TRACK POSITION
Portland State University
Department of Biology

Vertebrate Organismal Physiologists are invited to apply for a tenure track position at the Assistant or Associate Professor level. Comparative systems physiologists that are skilled in cellular and molecular approaches or cellular/molecular physiologists that appreciate the importance of higher levels of integration are equally acceptable. The quality of the research program is more important than the specific area of focus. The successful candidate will be expected to establish an independent research program that will attract extramural funding. The candidate will also be expected to contribute to the undergraduate curriculum, as well as provide research training for undergraduate and graduate students. An earned doctorate and an established research career are required.

Interested applicants should send current curriculum vitae, three letters of reference, a statement of research goals, and teaching philosophy and interests to:

Jason Podrabsky
Chair, Physiologist Search
Department of Biology
P.O. Box 751
Portland State University
Portland, OR 97207 0751

This position will remain open until filled; review of applications will begin December 1, 2005.

PSU is an Affirmative Action/Equal Opportunity Institution and, in keeping with the President's diversity initiative, welcomes applications from diverse candidates and candidates who support diversity.

FACULTY POSITIONS WITH TENURE at Yale University: The Department of Psychology at Yale University announces searches for two tenured Faculty Positions. One position in human neuroscience seeks candidates who can bridge across two or more of the core areas in the Department (behavioral neuroscience, clinical, cognitive, developmental, and social). The second position in cognitive psychology seeks candidates who can interact with the broader psychology department and also contribute to the interdisciplinary cognitive science program at Yale University. We invite applications from candidates who have international recognition for exceptional research, and who can also demonstrate excellence in teaching at both the undergraduate and graduate levels. All applicants should send a letter of application, curriculum vitae, one copy of selected publications, and the names and addresses of at least three referees. Materials should be sent to: **Chair, Senior Search in (please indicate either human neuroscience or cognitive psychology), Department of Psychology, Yale University, 2 Hillhouse Avenue, PO Box 208205, New Haven, CT 06520 8205.** Applications must be received by December 15, 2005. *Yale University is an Affirmative Action / Equal Opportunity Employer. Men and women of diverse racial/ethnic backgrounds and cultures are encouraged to apply.*

PROFESSOR IN AGROECOSYSTEMS MANAGEMENT AND CHARLES R. PARENCIA CHAIR IN COTTON ENTOMOLOGY. The Department of Entomology at Texas A&M University seeks qualified applicants for this endowed chair position with a 67 percent research and 33 percent teaching appointment. Applicants should possess a Ph.D. in entomology or a closely related biological science. Preference will be given to candidates with a strong background in the ecology, biology, and management of agroecosystems, particularly if related to cotton. The incumbent is expected to utilize a systems approach in addressing issues associated with cotton and inter related ecosystems, thus focusing on quantitative aspects of field-level and regional management. The incumbent will teach one section of Entomology 322 (Insects in Human Society) one semester each year and will develop a second undergraduate/graduate-level course taught one semester every other year. The incumbent will also have responsibility of mentorship and leadership training of undergraduate and graduate students. See details at **website: http://insects.tamu.edu** for full description and instructions for submission of application materials. Applicant review will begin December 7, 2005, and continue until a suitable candidate is selected. *Texas A&M University seeks individuals who are able to work with diverse students and colleagues, who have experience with a variety of teaching methods and curricular perspectives, and who will contribute to the diversity efforts of the University.*

IMMUNOLOGIST POSITION

St. Louis University, a Catholic Jesuit institution dedicated to student learning, research, health care, and service is seeking applicants for a tenure track Assistant Professor position in the Department of Biology. The successful candidate will work in Immunology or related disciplines and be expected to develop an independent, extramurally funded research program and to contribute to our undergraduate and graduate curricula. Excellent facilities and a competitive startup package are provided, and opportunities are available to collaborate with researchers at the nearby Health Science Center and local Universities. Applicants should have a Ph.D., post doctoral experience, and a record of research productivity. All applications must be made online at **website: http://jobs.slu.edu**; applications must include curriculum vitae and separate statements of teaching and research goals. Send reprints and three letters of recommendation by post to: **Dr. Richard L. Mayden, Department of Biology, St. Louis University, 3507 Laclede Avenue, St. Louis, MO 63103 2010.** Information about the Department and position is at **website: http://bio.slu.edu.** Review of applications will begin November 28, 2005, and will continue until suitable candidates are identified. *St. Louis University is an Affirmative Action, Equal Opportunity Employer, and encourages nominations and applications of women and minorities.*

FACULTY POSITION
Chemical Engineering, Princeton University

The Department of Chemical Engineering seeks outstanding applicants for a tenure-track position at the Assistant Professor level in the area of bioengineering, effective as early as July 1, 2006. The successful candidate should have a Ph.D. in chemical engineering or related field, demonstrated excellence in academic research, and a strong commitment to teaching and advising undergraduate and graduate students. Applicants should send curriculum vitae, a detailed description of teaching and research interests, reprints of selected publications, and the names and addresses of at least three references to: **Faculty Search Committee, Department of Chemical Engineering, Princeton University, Princeton, NJ 08544 5263.** Applications are encouraged before December 1, 2005. For information about applying to Princeton and how to self identify, please link to **website: http://web.princeton.edu/sites/dof/ApplicantsInfo.htm.** *Princeton University is an Equal Opportunity/Affirmative Action Employer. Women and minority candidates are encouraged to apply.*

POSITIONS OPEN

ASSISTANT PROFESSOR BIOLOGICAL SCIENCES: The Department of Biological Sciences and the Border Biomedical Research Center at the University of Texas at El Paso (UTEP) is seeking a tenure-track Assistant Professor starting July 2006, for its expanding research emphasis in toxicology. Candidates that focus on mechanisms of biochemical toxicology, pharmacology, or chemical carcinogenesis are particularly invited, although all areas of toxicology will be considered. In the fall of 2006, the Department **(website: http://academics.utep.edu/biology)** will occupy a new $30 million state of the art facility, which includes mammalian and aquatic animal facilities, and core facilities in tissue culture, molecular biology, protein chemistry, microscopy, and DNA sequencing. The successful candidate will develop and maintain a strong independent and extramurally funded research program and contribute to both undergraduate and graduate (M.S./Ph.D.) education.

Qualifications Required: Applicants must have a Ph.D. or M.D., and postdoctoral research experience.

Application Procedure: Applications should be sent to: **Dr. Lisa Bain, Department of Biological Sciences, The University of Texas at El Paso, 500 W. University Avenue, El Paso, TX 79968-0519,** and should include curriculum vitae, a statement of research interests, copies of three publications, and contact information for three references. Applications will be reviewed beginning December 15, 2005. *Equal Opportunity Employer.*

ASSISTANT PROFESSOR, BIOLOGICAL OCEANOGRAPHER
Florida State University

The Department of Oceanography is seeking applications for a Ph.D. level Biological Oceanographer for a nine-month, tenure-earning appointment at the Assistant Professor level to begin as soon as August 2006. We are particularly interested in a person who studies the role of zooplankton in ecological processes. The position involves research, teaching (primarily at the graduate level), and service. Please send a letter of application, curriculum vitae, and contact information for three references to: **The Biological Oceanography Search Committee, Florida State University, Department of Oceanography, Tallahassee, FL 32306-4320.**

Starting salary is negotiable, dependant upon qualifications, with a minimum salary of $59,000.

Closing date is December 1, 2005. Application review will begin on January 1, 2006. However, the advertising and search process will remain active until the position is filled.

An Equal Opportunity/Access/Affirmative Action Employer. The Florida State University subscribes to Equal Opportunity and complies with the Americans with Disabilities Act. All eligible candidates are invited to apply for position vacancies as appropriate. The Florida State University is a public records agency pursuant to Chapter 119, Florida Statutes.

The Section of Rheumatology seeks full-time academic faculty with demonstrated interest in basic, translational and/or clinical research in immunology and/or autoimmune diseases. The preferred candidate must have an MD, Ph.D. or MD/Ph.D. with training in clinical and/or laboratory based research. Excellent teaching skills are also required. M.D. candidates must be BC/BE in Rheumatology and must be eligible for medical licensure in the State of Illinois. Academic rank and salary commensurate with background and experience. Send curriculum vitae with references to:

Marcus Clark, M.D.
Chief, Section of Rheumatology
The University of Chicago
5841 South Maryland Avenue, (MC0930)
Chicago, IL 60637

The University of Chicago is an Affirmative Action/Equal Opportunity Employer.

POSITIONS OPEN

Western University of Health Sciences
The discipline of learning. The art of caring.

FACULTY POSITION
Pharmaceutical Biotechnology/ Pharmacogenomics
College of Pharmacy, Western University of Health Sciences

The Western University of Health Sciences, College of Pharmacy **(website: http://www.westernu.edu/cp.html)** invites applications for a tenure track Faculty Position in the Department of Pharmaceutical Sciences. A Ph.D. in pharmaceutical biotechnology or pharmacogenomics or a closely related discipline is required. The successful candidate will be expected to participate in the teaching activities of the professional (Pharm.D.) curriculum as well as the graduate program in the College. In addition, the candidate will be expected to establish and maintain an extramurally funded research program. Research startup funds are available to the successful candidate.

Rank and salary are negotiable, commensurate with qualifications and experience. Interested applicants should submit a letter of intent, a teaching and research statement, curriculum vitae, and arrange to have three letters of recommendation sent to the search committee chair. Electronic submissions of all appropriate materials are preferred and encouraged. Please send all application materials to: **Sunil Prabhu, Ph.D., Chair, Search Committee, College of Pharmacy, Western University of Health Sciences, College Plaza, 309 E. Second Street, Pomona, CA 91766-1854. Telephone: 909 469 5456. E mail: sprabhu@westernu.edu.**

Western University of Health Sciences is an Equal Opportunity/Affirmative Action Employer and actively seeks applications from women and minorities.

TENURE TRACK POSITION in biochemistry, Department of Chemistry and Center for Biomolecular Structure and Dynamics (CBSD), the University of Montana. The Department of Chemistry at the University of Montana seeks to fill a tenure track position in biochemistry. Research interests should be in biochemistry in areas that complement our strengths in environmental, organic, physical or analytical chemistry. The development of externally funded research programs, contributing to our undergraduate chemistry teaching and the development of upper division and graduate courses are required. The successful candidate will also become a member of the Graduate Program in Biomolecular Structure and Dynamics **(website: http://www.umt.edu/grad/programs/biomolecular/)**, and the associated CBSD, in addition to their tenure-track position in the Department of Chemistry. A Ph.D. in chemistry, biochemistry or a related field, postdoctoral experience and evidence of potential for teaching excellence at the undergraduate level are required. We seek to fill this position at the Assistant Professor level, but applications for the Associate level from candidates with outstanding records of scholarship and extramural funding are welcome.

Applications, including a complete curriculum vitae, a full description of future research plans, a summary of past research accomplishments and a statement of teaching philosophy should be sent to: **Nigel D. Priestley, Chair, Search Committee, Department of Chemistry, The University of Montana, Missoula, MT 59812-1656.** Applicants should arrange for three letters of recommendation to be sent to the same address. Information about the department can be accessed from our **website: http://www.umt.edu/chemistry.** The review of applications will begin on December 2, 2005, and continue until the position is filled. This position announcement can be made available in alternative formats upon request.

The University of Montana is an Equal Opportunity/Affirmative Action Employer, and encourages applications from women, minorities, veterans and persons with disabilities. The position is eligible for veterans' preference in accordance with State law.

POSITIONS OPEN

FACULTY OPENINGS IN NANOSCIENCE
Purdue University

The Purdue University College of Science has faculty openings in the broad area of nanoscience. This is part of a campus-wide emphasis on nanoscience and nanotechnology. This effort includes the Birck Nanotechnology Center, a new $60 million interdisciplinary facility, and approximately 20 new faculty positions that will be added in the Colleges of Engineering and Science over the next few years.

We seek exceptional faculty, with Ph.D., to complement and expand our existing expertise in all areas of experimental and theoretical nanoscience, but will give special emphasis to candidates with research interests in the areas of semiconductor nanostructures, advanced imaging at the nanometer scale especially as applied to biological systems, and computational nanoscience. Successful candidates will likely have a primary home department in physics, biology, chemistry, or computer science, and may have a joint appointment in another of these departments or in the College of Engineering at Purdue.

Candidates at all levels are encouraged to apply. Joint appointments across departments in the College of Science, or involving the Colleges of Science and Engineering, are anticipated.

For more information on the Purdue University College of Science, its areas of coalescence, and how to apply for a faculty position, visit our **website: http://www.science.purdue.edu/COALESCE/.** The review of applications will begin immediately, and continue until all of the positions are filled.

Purdue University is an Equal Opportunity/Equal Access/Affirmative Action Employer and is committed to building a diverse faculty of excellence.

ASSISTANT PROFESSOR, Neuroscience and Behavior, Department of Psychology, University of California at Santa Barbara. Applications are sought for a tenure track position beginning July 1, 2006. The Department is seeking a candidate who will contribute to the diversity and excellence of the academic community through research, teaching, and service. The Graduate Training Program in Neuroscience and Behavior is seeking a candidate in the area of neural development and/or plasticity whose research complements those within the department **(website: http://www.psych.ucsb.edu),** though strong applicants in all areas examining the relationship between brain and behavior will be considered. A Ph.D. in biopsychology or related discipline is required at the time of appointment. Please send curriculum vitae, statement of research and teaching interests, representative publications, and the names of three prospective references by November 15, 2005, to: **Chair, N and B Search Committee, Department of Psychology, University of California, Santa Barbara, CA 93106 9660.** *The University of California is an Equal Opportunity/Affirmative Action Employer.*

FACULTY POSITION
Chemical Engineering, Princeton University

The Department of Chemical Engineering seeks outstanding applicants for a tenure-track position at any rank in the area of Materials, effective as early as July 1, 2006. The successful candidate should have a Ph.D. in chemical engineering or related field, demonstrated excellence in academic research, and a strong commitment to teaching and advising undergraduate and graduate students. Applicants should send curriculum vitae, a detailed description of teaching and research interests, reprints of selected publications, and the names and addresses of at least three references to: **Faculty Search Committee, Department of Chemical Engineering, Princeton University, Princeton, NJ 08544 5263.** Applications are encouraged before December 1, 2005. For information about applying to Princeton and how to self identify, please link to **website: http://web.princeton.edu/sites/dof/ApplicantsInfo.htm.** *Princeton University is an Equal Opportunity/Affirmative Action Employer. Women and minority candidates are encouraged to apply.*

Midwestern State University
Dean of the College of Science and Mathematics

Midwestern State University invites nominations and expressions of interest for the position of Dean of the College of Science and Mathematics. The successful candidate will assume the position in summer 2006.

Midwestern State University, a Texas public university with about 6500 students is located in Wichita Falls (population 110,000), 120 miles northwest of Dallas. The College of Science and Mathematics, one of six colleges, serves approximately 700 undergraduate and 50 graduate students. Additional information can be found at **http://scienceandmath.mwsu.edu**.

Dr. Norman Horner, dean of the College, is retiring after many years of dedicated service to the University. His successor will find a cooperative College of about 50 faculty and staff members. His or her duties will include working with the chairs of the departments of Biology, Chemistry, Computer Science, Geosciences, Mathematics, and Physics, and the McCoy School of Engineering, and teaching up to 3 courses a year.

Applicants must have an earned doctorate in one of the College's disciplines; an established record of effective teaching and scholarly activity that warrants appointment as a full Professor in the College; academic, administrative, and fiscal management experience; knowledge of current trends and issues in higher education; commitment to diversity; and strong leadership and communication skills.

The position will remain open until an offer is made and accepted.

Qualified candidates should send a letter of application, that speaks to this advertisement, a current curriculum vitae, and a list, including phone numbers and e-mail addresses, of three references to: **Dr. Friederike Wiedemann, Provost, Midwestern State University, 3410 Taft Boulevard, Wichita Falls, TX 76308. E-mail: friederike.wiedemann @mwsu.edu**.

ADA/EEO

UCIrvine
SCHOOL OF BIOLOGICAL SCIENCES

ECOLOGY: TWO FACULTY POSITIONS

The Department of Ecology and Evolutionary Biology seeks to fill two tenure-track Assistant Professor positions in the area of Ecology. Possible areas of specialization include behavioral, population, community, and ecosystem ecology, without regard to taxon or system. Researchers studying any aspect of global biological change, including questions related to invasion, biodiversity, biogeography, land transformations and restoration, biogeochemistry, the effects of climate change, and conservation biology, are particularly encouraged to apply. Applicants interested in theory and modeling, as well as those working in the laboratory or field, will be considered. Each successful candidate will be expected to teach in undergraduate and graduate courses in ecology. Applications will be accepted until the positions are filled, but will be considered starting on **December 1, 2005**.

Please submit a letter of application including a statement of research and teaching interests, a curriculum vitae, and a sample of relevant publications, and arrange to have three letters of recommendation sent to: **Ecology Search Committee, Department of Ecology and Evolutionary Biology, 321 Steinhaus Hall, University of California, Irvine, CA 92697-2525**. The Department of Ecology and Evolutionary Biology (**http://ecoevo.bio.uci.edu/**) maintains strong ties with the Department of Earth System Sciences in the area of global change ecology (**http://globalchange.bio.uci.edu/;http://www.ess.uci.edu/**).

The University of California, Irvine has an active career partner program, is an Equal Opportunity Employer committed to excellence through diversity, and has a National Science Foundation ADVANCE Program in Gender Equity.

Tenure-track positions are open for outstanding individuals to establish research programs at Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan.

Applicants should have a Ph. D. degree and sufficient postdoctoral experience. Individuals with demonstrated records of research accomplishments and scientific creativity in all areas of **molecular and cellular biology** are strongly encouraged to apply. Junior scientists for the level of Assistant Research Fellow are most favorable. Senior members with excellent scientific performance are also welcome. Deadline for application is December 31, 2005.

Interested individuals should send Curriculum Vitae, a description of past research accomplishments and future research interests, and three letters of reference to:

Director's Office, c/o Fei Chen
Institute of Molecular Biology
Academia Sinica
Nankang, Taipei 115, Taiwan

Further information can be obtained from **Ms Fei Chen at feichen@ccvax.sinica.edu.tw** or from: **http://www.sinica.edu.tw/imb**

Faculty Position
Confocal/Multiphoton Microscopy
Medical University of South Carolina (MUSC)

The Department of Pharmaceutical Sciences at MUSC is searching for a tenure-track faculty member who uses confocal/multiphoton microscopy to study cell injury and death. The Department of Pharmaceutical Sciences is undergoing dramatic growth. Two new confocal microscopes (Zeiss LSM 510 NLO, CARVII) will arrive in January and will be housed in the Center for Cell Injury, Death and Regeneration in the new drug discovery building. Excellent opportunities exist for collaborations in the MUSC Hollings Cancer Center, Neuroscience Institute and Gazes Cardiac Research Institute. MUSC, located in the beautiful oceanside historic district of Charleston, receives over $180 million/year in research funding. Excellent salaries and benefits, competitive start-up packages and new laboratory space will be offered. The successful applicants will have a Ph.D. and postdoctoral experience and will be expected to develop an independent research program while participating in graduate and professional teaching.

Review of applications will begin **December 20, 2005** and will continue until the position is filled. Applicants should submit a curriculum vitae, statement of research interests, and contact information for four references to: **Dr. John J. Lemasters, c/o Sandy Spence, Department of Pharmaceutical Sciences, MUSC, 280 Calhoun St., POB 250140, Charleston, SC 29425; (843) 792-3117; spencesj@musc.edu**.

MUSC is an Equal Opportunity Employer.

NEUROSCIENCE CAREERS

THE UNIVERSITY OF FLORIDA COLLEGE OF MEDICINE

Chair, Department of Neuroscience

The University of Florida College of Medicine invites applications and nominations for the position of Professor and Chair in the Department of Neuroscience. The department has a mission within the college of both research and teaching responsibilities. The department has 24 primary faculty members with research interests in all aspects of neuroscience (**www.neuroscience.ufl.edu**). The department is housed within the McKnight Brain Institute (**www.mbi.ufl.edu**), a state-of-the-art facility for research and teaching in neuroscience and neuroscience-related disciplines. The successful candidate will have a Ph.D., M.D., or M.D./Ph.D. degree. We seek an accomplished scholar with a distinguished record of research, international recognition, leadership, and administrative skills.

The review of applications will begin on **December 1, 2005** and will continue until the position is filled. Applicants should provide a letter of application, curriculum vitae and the names of three references via email to **NS-search@phys.med.ufl.edu** or by mail to: **Charles E. Wood, Ph.D., Chair, Search Committee, Box 100274, Department of Physiology and Functional Genomics, University of Florida, Gainesville, FL 32610-0274**.

The University of Florida is an Equal Opportunity Institution.

POSITIONS OPEN

MOLECULAR BACTERIAL PATHOGENESIST
Colorado State University

The Department of Microbiology, Immunology, and Pathology is seeking outstanding bacterial pathogenesis candidates for a nontenure track faculty position at the Assistant Professor level as part of a campus wide Infectious Disease Initiative. The position involves a 95 percent commitment to research with 5 percent combined teaching and service. It is anticipated that the successful candidate will initially collaborate with established programs on the study of Mycobacterium tuberculosis within the Mycobacteria Research Laboratories (MRL) leading to an independent research program in infectious diseases. This individual is expected to build a research program on molecular mechanisms of bacteria host interactions leading to a physiological understanding of infectious disease processes. A Ph.D., M.D./Ph.D., or D.V.M./Ph.D. in the area of molecular bacterial pathogenesis and a minimum of three years of postdoctoral training are required.

Qualified individuals should submit applications by mail or e-mail. Complete applications, including a cover letter; current curriculum vitae; the names, addresses, and e-mail addresses of three references; and a short description of research interests to:

Erin Napier
Colorado State University
Department of Microbiology, Immunology, and Pathology
1619 Campus Delivery
Fort Collins, Colorado 80523 1619
Fax: 970-491-0
E mail: erin.napier@colostate.edu.

Evaluation of applications will begin December 15, 2005, and will continue until the position is filled. *Colorado State University is an Equal Opportunity Employer.*

BRADLEY UNIVERSITY

The Department of Biology invites applications for a tenure track position in cell biology/molecular biology/developmental biology at the Assistant Professor level to begin fall 2006. A Ph.D. is required and postdoctoral experience is preferred. The successful candidate will be expected to teach cell and molecular biology, courses in the area of specialization, and general biology courses. In addition, the candidate will be expected to establish a productive, externally funded research program in the area of expertise, and to contribute to the broader biology curriculum.

Applicants must demonstrate a strong commitment to undergraduate teaching and research, and the promise of scholarly and pedagogical excellence in a liberal arts setting. Submit a letter of application, curriculum vitae, graduate and undergraduate transcripts, statements of teaching interests and research plans, and three letters of reference to: **Search Committee, Biology Department, Bradley University, Peoria, IL, 61625.** Applications received by December 15, 2005, will receive first consideration. For a full position description, please visit **website: http://www.bradley.edu/humanresources.**

Bradley University is an Equal Opportunity and Affirmative Employer. The administration, faculty and staff are committed to attracting qualified candidates from groups currently underrepresented on our campus.

BENTHIC MARINE ECOLOGIST

The Marine Laboratory at the Sanibel Captiva Conservation Foundation seeks a full-time Benthic Marine Ecologist (Ph.D., or M.S. with experience) with a specialty is in seagrass or microbial community ecology. More details regarding the Foundation (**website: http://www.sccf.org**) and the position are on our website. Applicants should send a letter of interest, detailed resume, and contact information for at least three references to: **Dr. Stephen A. Bortone, Marine Laboratory Director, Sanibel-Captiva Conservation Foundation, 900A Tarpon Bay Road, Sanibel, Florida 33957 U.S.A.**

POSITIONS OPEN

POSTDOCTORAL AND RESEARCH ASSOCIATE POSITIONS
Keck Graduate Institute of Applied Life Sciences

Available immediately to develop cell based assay for drug screening. Successful candidates must be experienced in tissue culture, molecular and cellular biology. Knowledge in enzyme kinetics desirable. Good communication skills, problem solving skills, ability to work independently and self-motivation are expected. Keck Graduate Institute (**website: http://www.kgi.edu**) is a member of The Claremont Colleges, located about 30 miles east of Los Angeles. To apply, please send curriculum vitae and names and addresses of three professional references to:

Dr. Chen-Chen Kan
Keck Graduate Institute
535 Watson Drive
Claremont,CA 91711
Fax: 909-607-8086
E mail: Chen Chen_Kan@kgi.edu.

Biomolecular Nuclear Magnetic Resonance Spectroscopy
Texas A&M University

The Department of Biochemistry and Biophysics at Texas A&M University invites applications for a **FACULTY POSITION** in the area of macromolecular nuclear magnetic resonance (NMR) spectroscopy. Individuals investigating the structure and function of macromolecular complexes (proteins and nucleic acids) and membrane proteins are particularly encouraged to apply. Outstanding candidates at both the tenure-track Assistant Professor and Associate Professor levels will be considered. Texas A&M University is committed to building in the area of structural biology, with efforts underway to add an 800 MHz instrument to lower field instruments (500/600s) currently available. The successful candidate will join a strong interdisciplinary core of structural biologists and become a faculty trainer in our NIH-supported Molecular Biophysics Training Program. In addition to establishing and maintaining a vigorous independent research program, the successful candidate will be committed to teaching at both the undergraduate and graduate student levels. Candidates should submit curriculum vitae, a brief description of research plans (not to exceed five pages), and arrange for three letters of recommendation to be sent to: **NMR Spectroscopy Search Committee, Texas A&M University, Department of Biochemistry and Biophysics, 2128 TAMU, College Station, TX 77843 2128.** Review of applications will begin December 1, 2005, and will continue until the position is filled. *Texas A&M University is an Equal Opportunity/Affirmative Action Employer committed to diversity.*

SCIENCE ANALYST

Join the scientific department of a New York City law firm and help us to identify, summarize and critically evaluate biological and medical research literature and statistical data relevant to product liability litigation. We are seeking expertise in the molecular and cellular biology of cancer. Good communication skills – both written and oral – are required to present analyses of medical and scientific issues to nonscientists. Must be a team player and a self-starter with the ability to set priorities and meet deadlines. Doctoral degree preferred. Experience in a nonacademic position (consulting, industry, litigation, etc.) a plus. This position does not involve appearances in court. Competitive salary and excellent benefits. Send resume, publication list (if applicable), salary history and requirements to: **Ms. Zweifler at: e mail: fdzweifler@jmfnylaw.com, or fax: 212-524-5050.**

POSITIONS OPEN

The Chinese University of Hong Kong, Department of Physics, invites applications for the post of Professor (Ref. 05/169(665)/2). Applicants should have (1) a doctoral degree in Experimental Physics or Materials Science or related discipline(s); (2) an outstanding record of research accomplishments; and (3) the ability to lead a visible and significant research programme. Potential for interaction with faculty and researchers in semiconductor physics, surface science, optics, and nanostructure materials will be considered favorably. Appointment will normally be on a fixed term contract basis for up to three years, with prospect for a longer-term appointment thereafter subject to mutual agreement. Exceptionally, substantive appointment can be offered forthwith to candidates of proven ability. For enquiries, please **e mail: physics@cuhk.edu.hk.** Monthly salary ranges from HK$76,435 – HK$93,810 [Approximate exchange rate in October 2005: US$1 ~ HK$7.8]. The position is open till filled. Salary will be highly competitive, commensurate with qualifications and experience. The University offers a comprehensive fringe benefits package, including medical care, plus a contract-end gratuity for an appointment of two years or longer and housing benefits for eligible appointees. Further information about the University and the general terms of service for appointments is available at **website: http://www.cuhk.edu.hk/personnel.** The terms mentioned herein are for reference only and are subject to revision by the University. Please send full resume, copies of academic credentials, a publication list and/or abstracts of selected published papers, statement of research interests and proposed directions, together with names, addresses and fax numbers/e mail addresses of three references to whom the applicants' consent has been given for their providing references (unless otherwise specified), to: **Chairman, Department of Physics, Science Centre, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong,** or by **fax: 852-2603 5204.** The Personal Information Collection Statement will be provided upon request. Please quote the reference number and mark "**Application – Confidential**" on cover.

TENURE TRACK POSITION
University of North Carolina, Chapel Hill
Marine Ecology/Biological Oceanography

The Marine Sciences Department invites applications to fill a faculty position at Assistant Professor level. We seek applications from candidates in any area of marine ecology or biological oceanography whose work integrates with existing strengths in both the life and Earth sciences, and who apply experimental, quantitative, descriptive or molecular techniques to solve cutting edge, interdisciplinary problems. The successful candidate for this position will have the potential to fund and perform high quality research and teach effectively at the graduate and undergraduate levels. The position carries nine months of salary support. For further information see **website: http://www.marine.unc.edu.** Submit curriculum vitae, and statements of research and teaching interests to: **Chair, Marine Ecology/Biological Oceanography Search, Department of Marine Sciences, 12-7 Venable Hall CB 3300, Chapel Hill NC 27599.** Arrange to have four letters of recommendation sent to the same address. Review of applications will begin on December 1st 2005, but the search will continue until the position is filled. *The University of North Carolina is an Equal Opportunity Employer.*

POSTDOCTORAL FELLOWSHIPS, University of Southern California (USC): NIA funded training programs are accepting applications for fellowships in: Neurobiology and Endocrinology of Aging, **Caleb Finch, Ph.D., P.I.,** Contact: **Lisbeth Ruiz, e-mail: lruiz@usc.edu;** and Multidisciplinary Research Training in Gerontology, **Eileen Crimmins, Ph.D., P.I.** Contact: **Linda Hall, e-mail: lindah@usc.edu.** Immediate openings and recruiting for 2006/2007. Must be U.S. citizen or hold current I551.

POSITIONS OPEN

ASSISTANT PROFESSIONAL SCIENTIST LARGE-RIVER ECOLOGIST

The Center for Aquatic Ecology and Conservation (CAEC) at the Illinois Natural History Survey (INHS) is accepting applications for an ecologist who works on large rivers. The position is at the level of Assistant Professional Scientist, and the successful candidate will direct the Illinois River Biological Station (IRBS) and develop an externally funded research program. Professional scientists within CAEC conduct self directed research, have full access to UI campus facilities and resources, hold adjunct or affiliate positions in University departments, teach courses, and supervise graduate students. The IRBS in Havana, IL, is one of six field stations associated with the Long Term Resource Monitoring Program. Facilities at the IRBS include wet and dry laboratories, offices, research boats, vehicles, and river sampling equipment. To qualify for this position, candidates must possess a doctorate in aquatic ecology or a related discipline and have had research experience in large rivers or other large freshwater ecosystems. Candidates with postdoctoral research experience who desire to work on interdisciplinary projects with scientists inside and outside of the INHS are preferred. For full position announcement and application instructions see **website: http://www.inhs.uiuc.edu/opportunities/index.html**. Direct technical questions to: **Dr. John Chick, telephone: 618 466 9690. E mail: chick@inhs.uiuc.edu.** Deadline for application: December 1, 2005. Start date: August 1, 2006. *INHS is an Equal Opportunity Employer.*

TENURE TRACK ASSISTANT PROFESSOR Terrestrial Animal Ecology

The Department of Zoology, University of British Columbia, seeks applications for a Tenure-Track Position in terrestrial animal ecology. The successful applicant is expected to develop a strong research program, teach courses in ecology or organismal biology and actively participate in the UBC Biodiversity Research Centre.

Salary will be commensurate with experience. Appointment will be at the Assistant Professor level and is subject to final budgetary approval.

Applicants should send a curriculum vitae, summary of research interests and teaching philosophy, and reprints of three key publications. Letters should be sent directly from three referees. Address all materials to: **Dr. Bill Milsom, Head, Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, BC, Canada, V6T 1Z4. E-mail: head@zoology.ubc.ca, fax: 604-822-5780.** Deadline for applications is 15 December 2005, or until a suitable candidate is found.

All qualified applicants are encouraged to apply; however, Canadian citizens and permanent residents of Canada will be given priority. The University of British Columbia hires on the basis of merit and is committed to employment equity.

TENURE TRACK ASSISTANT PROFESSOR Biomedical Science

Applications are encouraged from candidates with a doctorate and postdoctoral experience in an area of Biomedical Science such as neurobiology, endocrinology, developmental biology, cancer biology, pathology or related field. Successful candidates are expected to participate in undergraduate and graduate teaching and to develop an externally funded research program. Send letter of application, academic transcripts, statements of research interests and teaching philosophy, vitae, and names and contact information for five references to:

Cynthia Mondragon
School of Biological Sciences
Ross Hall, Box 92
University of Northern Colorado
Greeley, CO 80639

For details see **website: http://www.unco.edu/nhs/employment/.** For questions contact **e-mail: .** Review of applications begins January 10, 2006. *University of Northern Colorado is an Affirmative Action, Equal Opportunity Employer.*

POSITIONS OPEN

DES MOINES UNIVERSITY

FACULTY POSITION IN ANATOMY

The Department of Anatomy at Des Moines University-Osteopathic Medical Center invites applications for a 12 month, tenure track faculty position at the Assistant or Associate Professor level. The successful candidate shall have demonstrated teaching excellence in the anatomical sciences, primarily human gross anatomy, embrace the application of effective integration of technology in the educational process, and develop and sustain an extramurally funded research program. Applicants must have a doctorate degree and postdoctoral experience. Position is available immediately. For best consideration apply by January 15, 2006. Submit a letter of application, curriculum vitae, statements of teaching philosophy and research interests, and contact information for three references to:

Human Resources
Des Moines University-Osteopathic Medical Center
3200 Grand Avenue
Des Moines, Iowa 50312-4198
E mail: employment@dmu.edu
Website: http://www.dmu.edu

MICROBIOLOGIST ORGANISMAL BIOLOGIST

The Department of Biology at the University of West Georgia **(website: http://www.westga.edu/~biology)** invites applications for two tenure-track faculty positions at the rank of Assistant Professor. A doctoral degree and record of research productivity in the discipline and/or in pedagogy are required; postdoctoral training and teaching experience are preferred. Teaching responsibilities include participation in the required core for undergraduate biology majors or nonscience majors and advanced undergraduate and graduate biology courses in specialty area. Position 1, Microbiologist: area of specialization is open, although qualification to teach environmental microbiology and/or microbial physiology is preferred. Position 2, Organismal Biologist: area of specialization is open but qualification to teach behavioral biology is preferred. Please submit a letter of application, curriculum vitae, statements of teaching philosophy and research interests/plans, copies of transcripts, and arrange for three current letters of recommendation to be sent to: **Dr. Leos Kral (Microbiology position)** or **Dr. Greg Payne (Organismal Biology position), Department of Biology, University of West Georgia, Carrollton, GA 30118.** Submissions in PDF format may be sent to **e mail: biosearch@westga.edu.** Review of applications will begin November 18, 2005, with an anticipated start date of August 2006. *UWG is an Equal Opportunity/Affirmative Action Employer.*

RESEARCH SCIENTIST POSITION Stable Isotope Ecology/Geochemistry

The University of Wyoming Stable Isotope Facility seeks a research scientist and facility manager. Minimum qualifications: Ph.D. with demonstrated research experience (publications, grants) utilizing stable isotopes for environmental or paleoenvironmental research, experience managing an isotope lab, and at least two years of experience operating stable isotope ratio mass spectrometers. This is a permanent, full time (twelve month) appointment as an Academic Professional Research Scientist with a starting date of November 28, 2005. Applicants should submit curriculum vitae, letter of interest, and names and contact information for three references to:

Dr. David G. Williams (e-mail: dgw@uwyo.edu)
Department of Renewable Resources
University of Wyoming
Laramie, WY, 82071

Review of applications will begin on November 14, 2005. The University of Wyoming is an Equal Opportunity/Affirmative Action Employer. Full job announcement at **website: http://uwadmnweb.uwyo.edu/hremployment2/Nonclass.htm#RS4637.**

POSITIONS OPEN

ARID LANDS ECOLOGIST Colorado State University

Assistant Professor, tenure-track, nine-month academic faculty position; Department of Forest, Rangeland and Watershed Stewardship. The Department of Forest, Rangeland, and Watershed Stewardship at Colorado State University (CSU) invites applications and nominations for an **ASSISTANT PROFESSOR** in the ecology, management, or conservation of arid and semiarid lands. A complete job description and general information about the Department can be found at **website: http://www.warnercnr.colostate.edu/frws/.** Qualifications: Required: (1) Ph.D. in rangeland ecology/science, landscape ecology, plant community ecology, conservation biology, or closely related field; (2) expertise in the ecology, management, or conservation of arid and semiarid lands relevant to goals for sustainable production. Desirable: (1) a graduate or undergraduate degree in rangeland ecology/science; (2) teaching experience; (3) refereed publications; (4) experience in conducting research at the landscape scale; (5) postdoctoral experience; (6) ability to involve stakeholders in research; (7) ability to communicate scientific knowledge to practitioners, managers, and policy makers; (8) experience working in interdisciplinary teams; (9) interest and/or experience working in the western United States and internationally.

For application information and a detailed job description please log on to **website: http://www.warnercnr.colostate.edu/hr/.** *CSU is an Equal Opportunity/Affirmative Action Employer.*

ASSISTANT PROFESSOR BIOLOGICAL OCEANOGRAPHER

The Department of Biology and Marine Biology at the University of North Carolina, Wilmington (UNCW) invites applications for a tenure-track position starting in August 2006. Candidates in any subdiscipline of biological oceanography may apply. Duties include undergraduate and graduate teaching, maintaining an active research program, and directing graduate students. The Department offers B.S., M.S., and Ph.D. degrees in marine biology and other degrees. A modern science building and the Center for Marine Science offer excellent support for marine research (**websites: http://www.uncw.edu/bio and http://www.uncw.edu/cmsr**). Candidates must have a Ph.D. and postdoctoral experience. To apply, complete the online application process available at **website: http://consensus.uncw.edu.** A letter of application including brief statements of teaching and research interests, curriculum vitae, and contact information for three references should be addressed to **Dr. Lawrence Cahoon, Oceanography Search Committee,** and attached to the online application, not e-mailed or mailed. Microsoft Word or Adobe PDF attachments are preferred. For questions about the online application process, contact: **Ms. Debbie Cronin, telephone: 910-962-3707.** Screening of applications will begin January 10, 2006. Under North Carolina law, applications and related materials are confidential personnel documents and not subject to public release. *UNCW is an Equal Opportunity/Affirmative Action Employer. Women and minorities are encouraged to apply.*

POSTDOCTORAL FELLOW

A Postdoctoral Fellow position is open in the laboratory of **Dr. Brian B. Haab** to study cancer-related alterations in specific blood proteins. We are particularly interested in the effect of oxidative stress on the glycan composition of secreted proteins, which we address using novel, multiplexed, protein analysis methods. The position requires a Ph.D. in a life sciences discipline or an M.D. with a research focus. Qualified candidates should submit curriculum vitae, a brief cover letter describing interests and goals, and the names and addresses of three references to **e mail: vari employment@vai.org** or mail to: **Van Andel Institute, Human Resources - Req. 589, 333 Bostwick NE, Grand Rapids, MI 49503.** *Equal Opportunity Employer*

THE 2006 LOUISA GROSS HORWITZ PRIZE FOR BIOLOGY OR BIOCHEMISTRY

COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK

The Louisa Gross Horwitz Prize was established under the will of the late S. Gross Horwitz through a bequest to Columbia University and is named to honor the donor's mother. Louisa Gross Horwitz was the daughter of Dr. Samuel David Gross (1805–1889), a prominent surgeon of Philadelphia and author of the outstanding *Systems of Surgery*, who served as president of the American Medical Association.

Each year since its inception in 1967, the Louisa Gross Horwitz Prize has been awarded by Columbia University for outstanding basic research in the fields of biology or biochemistry. The purpose of this award is to honor a scientific investigator, or group of investigators, whose contributions to knowledge in either of these fields is deemed worthy of special recognition.

The Prize consists of an honorarium and a citation which are awarded at a special presentation event. Unless otherwise recommended by the Prize Committee, the Prize is awarded annually. Dr. Ada Yonah of the Weizmann Institute of Science, Rehovot, Israel, was the 2005 awardee.

QUALIFICATIONS FOR THE AWARD

The Prize Committee recognizes no geographical limitations. The prize may be awarded to an individual or a group. When the prize is awarded to a group, the honorarium will be divided among the recipients, and each member will receive a citation. Preference will be given to work done in the recent past.

Prospective recipients should be nominated electronically at: http://cumc.columbia.edu/horwitz/

Electronic nominations should include:

1. A summary, preferably less than 500 words, of the research on which this nomination is based.
2. A summary, preferably less than 500 words, of the significance of this research in the fields of biology or biochemistry.
3. A brief biographical sketch of the nominee, including positions held and awards received by the nominee.
4. A listing of up to ten of the nominee's most significant publications relating to the research noted under item 1.
5. A copy of the nominee's curriculum vitae.

Nominations must be submitted no later than **January 21, 2006.**

Children's Tumor Foundation
Ending Neurofibromatosis Through Research

NEUROFIBROMATOSIS YOUNG INVESTIGATOR AWARDS 2006

Neurofibromatosis (NF) encompasses neurofibromatosis 1, neurofibromatosis 2 and schwannomatosis, disorders that cause potentially devastating neurological tumors and an array of complications including deafness, vision loss and learning disabilities. NF affects an estimated 100,000 persons in the US; can seriously impact on health and quality of life; and can be fatal.

Children's Tumor Foundation is committed to ending neurofibromatosis through research. The Foundation offers the Young Investigator Awards to encourage pre-doctoral and postdoctoral researchers (no more than four years after completion of doctoral training) in the neurosciences and other disciplines to focus their career on neurofibromatosis related research. Areas of research relevant to NF include, but are not limited to, tumor signal transduction pathways; cell lineage and cell differentiation; molecular genetics; mechanisms and management of pain; and cognitive function. Applications are welcomed that address basic research, translational or clinical studies.

Awards provide salary support commensurate with NIH-equivalent level of training, up to $45,000/year for up to two years. Financial support for attendance at the Foundation's annual NF Consortium and other relevant meetings or training courses will be available.

For a full description of this program see our 2006 Request for Applications at **http://www.ctf.org/professionals/yia.htm**. Or visit our booth **(#1024)** at SFN 2006

2006 APPLICATIONS: Application materials will be available January 1, 2006 at **www.ctf.org**. Intent to submit application (first 2 pages) due: **February 14, 2006.** Full application due: **April 1, 2006.**

For further information contact:
Ms. Cicely Acosta, Grants Administrator
Children's Tumor Foundation
Tel: 212-344-6633 or email: cacosta@ctf.org

POSITIONS OPEN

FACULTY POSITION
X-Ray Crystallography
Department of Biochemistry and Molecular Biology
University of Oklahoma Health Sciences Center

The Department of Biochemistry and Molecular Biology at the University of Oklahoma Health Sciences Center (**website: http://w3.ouhsc.edu/biochem/**) invites applications for a tenure track position at the level of Assistant or Associate Professor. Candidates should have a Ph.D., MD, or equivalent degree, and significant postdoctoral experience. Candidates with an established track record and extramural funding will be considered at the Associate Professor level or higher. The successful candidate will use X-ray crystallography as a primary research tool to investigate questions of biological importance. The candidate will develop a well-funded research program and have a major role in the direction of the Laboratory for Macromolecular Crystallography (**website: http://w3.ouhsc.edu/biochem/Crystallography/index.htm**). In addition to the crystallography laboratory, which has recently been upgraded, the Department has core facilities in mass spectrometry, glycobiology, and biophysics. The Department has 18 full time and ten adjunct faculty, who maintain active and well funded research programs. There is an active crystallography community on campus that includes members of the Oklahoma Medical Research Foundation (OMRF). The University of Oklahoma (OU) Health Sciences Center and the OU Medical Center, comprised of seven colleges, four hospitals, the Presbyterian Health Foundation Research Park, and the OMRF, with over 2200 faculty, is located on a 200 acre modern campus. Applications should include a cover letter, curriculum vitae, outline of current and future research, contact information for three references, and up to five papers. The Search Committee will begin reviewing applications November 21, 2005, and will continue until the position is filled. Applications should be sent as a single PDF to **e-mail: Xtal Search@ouhsc.edu,** using Crystallography Search Committee as the subject line. *The University of Oklahoma is an Equal Opportunity/Affirmative Action employer.*

A **POSTDOCTORAL POSITION** is available on Duchenne muscular dystrophy gene therapy. Applicants must hold a doctoral degree and have a strong background in molecular biology and/or physiology. Please send curriculum vitae and the names of three references to:

Dongsheng Duan, Ph.D.
Molecular Microbiology and Immunology
M616 Medical Sciences Building
University of Missouri
Columbia, MO 65212
E mail: duand@missouri.edu
Website: http://www.missouri.edu/~mmiwww/dongsheng/dd.php

To request ADA accommodations, please call ***telephone: 573-884-7278 (V/TTY).***

The Section of Brain Tumor Biology, Department of Neurosurgery, Brain Tumor Center of Excellence (BTCOE), of the Comprehensive Cancer Center, Wake Forest University School of Medicine is recruiting a **POSTDOCTORAL FELLOW**. The candidate should have strong interest in studying brain tumors and expertise in one or more of the following areas: molecular pharmacology, molecular biology and/or biochemistry. The position is NIH-funded and the starting date is flexible. Send curriculum vitae, statement of research interests and two references to: **Waldemar Debinski, M.D., Ph.D.; e mail: debinski@wfubmc.edu.**

SENIOR SCIENTIST (New Jersey): Oversee analytical method development and validation for new products (Abbreviated New Drug Application or New Drug Application). Required: M.S. or equivalent and five years of experience. Send resume to: **Human Resources, Alpharma, 200 Elmora Avenue, Elizabeth, NJ 07207, Attn: Ellen.** No calls.

TWO ASSISTANT PROFESSOR POSITIONS
CELL BIOLOGIST AND VERTEBRATE BIOLOGIST

The Department of Biology and Marine Biology at the University of North Carolina Wilmington invites applications for two tenure track positions starting August 2006.

Cell Biologist: Candidates in any subdiscipline of eukaryotic cell biology are encouraged to apply.

Vertebrate Biologist: Candidates with an interest in integrative and comparative biology are encouraged to apply.

Duties for both positions include undergraduate and graduate teaching, and maintaining an active research program that involves both graduate and undergraduate students. The Department offers a B.A. in biology, B.S. and M.S. degrees in biology and in marine biology, and a Ph.D. in marine biology. Modern laboratories and diverse core facilities are available in the Department and at the Center for Marine Science (**websites: http://www.uncw.edu/bio/** and **http://www.uncw.edu/cmsr/**). Candidates must have a Ph.D. and postdoctoral experience. To apply, complete the online application available at **website: http://consensus.uncw.edu.** The application package should include a letter of interest that must contain brief statements of teaching and research interests, curriculum vitae, and contact information for three references. Microsoft Word and Adobe PDF documents are the preferred programs for attachments. The Chair of the Cell Biologist search is **Dr. Stephen Kinsey (telephone: 910 962 7398),** and the Chair of the Vertebrate Biologist search is **Dr. D. Ann Pabst (telephone: 910 962 7266),** Department of Biology and Marine Biology. For questions about the online application process, contact **Ms. Tracie Chadwick (telephone: 910 962 3536).** Application review will begin January 10, 2006. Under North Carolina law, applications and related materials are confidential personnel documents and not subject to public release. *UNCW is an Equal Opportunity/Affirmative Action Employer. Women and minorities are encouraged to apply.*

ASSISTANT PROFESSOR: Biochemistry, Chemistry and Physics Department at Niagara University invites applications for a tenure-track faculty position in biochemistry (fall 2006). Ph.D. in biochemistry, preferable; successful postdoctoral experience, strong potential to develop an externally funded research program, demonstrate commitment to teaching at undergraduate level, and strong oral/written communication skills. Responsibilities: provision of high quality teaching and scholarship, mentoring students, supervising practicums, and serving on departmental/college committees. Department is currently expanding and wishes to hire faculty that will encourage that growth. With many new state-of-the art facilities, the successful candidate will have plenty of opportunity to develop a strong research platform and will be part of the newly founded Niagara University Academic Center for Integrated Sciences, which encourages collaborations with other departments as well as local industry. Initial start-up funds will be provided. Located near the scenic Niagara Falls, Niagara University is a predominantly undergraduate liberal arts university in the Catholic Vincentian tradition. Application letter, curriculum vitae, three letters of recommendation, teaching philosophy, and research plan to: **Dr. Mary McCourt, Chairperson, Chemistry, Biochemistry, and Physics Department, Niagara University, NY 14109-2044.** Applications reviewed until position filled. *Affirmative Action/Equal Opportunity Employer.*

A **POSTDOCTORAL POSITION** is available to study the structure and function of Alzheimer's amyloid precursor protein (*Molecular Cell* 15:343–353, 2004). Experiences in protein chemistry and nuclear magnetic resonance are required. Please send curriculum vitae, a brief description of career goals, and names of three references to: **Dr. Ya Ha, 333 Cedar Street, New Haven, CT 06520, U.S.A. E-mail: ya.ha@yale.edu.**

POSTDOCTORAL POSITIONS are available at George Mason University for two Computational Neuroscientists and one Experimental Neuroscientist beginning in January 2006. Two positions are funded by the National Institutes of Health to study dopamine activated second messenger pathways in the striatum in collaboration with Dietmar Plenz; one position is funded by the Human Frontiers Science Program to study the role of A kinase anchoring proteins and second messenger pathways in hippocampal synaptic plasticity. The experimental neuroscientist should have a Ph.D. in neuroscience or a related field and electrophysiology or imaging skills. The computational neuroscientists should have a Ph.D. in neuroscience or a quantitative field (math, physics, engineering) and C programming or neural modeling skills. Salary $35,000. For further details see **website: http://www.gmu.edu/departments/krasnow/CENlab/CENlab.html.** Review of applications will begin December 1, 2005, and continue until filled. Send curriculum vitae, a brief description of your motivation, and references to: **Kim Blackwell, V.M.D., Ph.D., Krasnow Institute for Advanced Study, M.S. 2A1, George Mason University, Fairfax, VA 22030;** or **e mail: avrama@gmu.edu.** *Affirmative Action/Equal Opportunity Employer.*

RESEARCH SCIENTIST: Seeking Physicist with background in mathematics to work in human neurophysiology laboratory on brain dynamics. Knowledge of signal processing desirable and knowledge of statistics a plus. Experience with UNIX and Windows. Knowledge of at least one technical programming language and two scripting languages required. Minimum M.S. required; Ph.D. desirable. Salary range $50,000 to $60,000. Excellent benefits. Please send curriculum vitae to: **Bernice Porjesz/Henri Begleiter, Department of Psychiatry, State University of New York Downstate Medical Center, P.O. Box 1203, 450 Clarkson Avenue, Brooklyn, NY 11203,** or **fax: 718 270 4081.** *Equal Opportunity Employer.*

POSTDOCTORAL POSITION: Position available to study the pathophysiology of the septo hippocampal system. The laboratory work is focused in understanding septal neuronal networks and their role in abnormal excitability states including Alzheimer's disease. The position requires expertise in electrophysiology. Skills in immunohistochemistry, tissue culture and molecular biology are desirable. Please send curriculum vitae, summary of research interests and three letters of reference to: **Luis V. Colom (e-mail: luis.colom@uth.edu), Department of Biological Sciences, The University of Texas at Brownsville, 80 Fort Brown, Brownsville, Texas 78520.**

POSTDOCTORAL RESEARCH POSITIONS
Biological/Social Psychiatry
Harvard Medical School

Research training in clinical neuroscience and developmental psychopathology, including family studies with integrative seminar. For Ph.D.s and M.D.s who are U.S. citizens or permanent residents. Reviews begin January 2, 2006. Contact: **Clinical Research Training Program, Judge Baker Children's Center, 53 Parker Hill Avenue, Boston, MA 02120 3225. Telephone: 617 232 8390, extension: 4293; e-mail: crtp@jbcc.harvard.edu.** *Minority applications encouraged.*

The Arnold Arboretum of Harvard University announces the availability of **MERCER AND PUTNAM POSTDOCTORAL FELLOWSHIPS.** Applications are due December 1, 2005. Support of a sponsoring faculty member or senior researcher at the University is essential. Information and application instructions can be obtained at website: **http:// www.arboretum.harvard.edu/research/research awards.html.**

Cold Spring Harbor Laboratory
2006 Meetings & Courses

Meetings

Neuronal Circuits: From Structure To Function
March 9 - 12

PTEN Pathways
March 15 - 19

Systems Biology: Global Regulation of Gene Expression
March 23 - 26

Channels Receptors & Synapses
April 18 - 22

Gene Expression and Signalling in the Immune System
April 26 - 30

Molecular Chaperones & the Heat Shock Response
May 3 - 7

The Biology of Genomes
May 10 - 14

The Cell Cycle
May 17 - 21

Retroviruses
May 23 - 28

71st Symposium: Regulatory RNAs
May 31 - June 5

Glia in Health & Disease
July 20 - 24
Ben Barres, Martin Raff

Mechanisms & Models of Cancer
August 16 - 20

Molecular Genetics of Bacteria & Phages
August 23 - 27

Mouse Molecular Genetics
August 30 - September 3

Translational Control
September 6 - 10

Axon Guidance, Synaptogenesis & Neural Plasticity
September 13 - 17

Dynamic Organization of Nuclear Function
September 27 - October 1

Molecular Genetics of Aging
October 4 - 8

Germ Cells
October 11 - 15

Nuclear Receptors & Disease
November 2 - 5

Pharmacogenomics
November 15 - 18

Neurodegenerative Diseases: Biology & Therapeutics
November 30 - December 3

http://meetings.cshl.edu

Courses

Protein Purification & Characterization
March 29 - April 11

Cell & Developmental Biology of *Xenopus*
April 1 - 11

Genetics of Complex Human Diseases
June 7 - 13

Advanced Bacterial Genetics
June 7 - 27

Ion Channel Physiology
June 7 - 27

Molecular Embryology of the Mouse
June 7 - 27

Integrated Data Analysis for High Throughput Biology
June 14 - 27

Computational Neuroscience: Vision
June 16 - 29

Advanced Techniques in Plant Science
June 30 - July 20

Neurobiology of *Drosophila*
June 30 - July 20

Mechanisms of Neural Differentiation & Brain Tumors
July 6 - 12

Advanced Techniques in Molecular Neuroscience
July 6 - 20

Proteomics
July 7 - 20

Eukaryotic Gene Expression
July 25 - August 14

Imaging Structure & Function in the Nervous System
July 25 - August 14

Yeast Genetics & Genomics
July 25 - August 14

C elegans
July 27 - August 14

Stem Cells & Regeneration
August 3 - 16

X-Ray Methods in Structural Biology
October 16 - 31

Programming for Biology
October 18 - 31

Immunocytochemistry, In Situ Hybridization & Live Cell Imaging
October 18 - 31

Phage Display of Proteins & Peptides
November 7 - 20

Computational & Comparative Genomics
November 8 - 14

Above: Weekend sailing off the Cold Spring Harbor Laboratory beach

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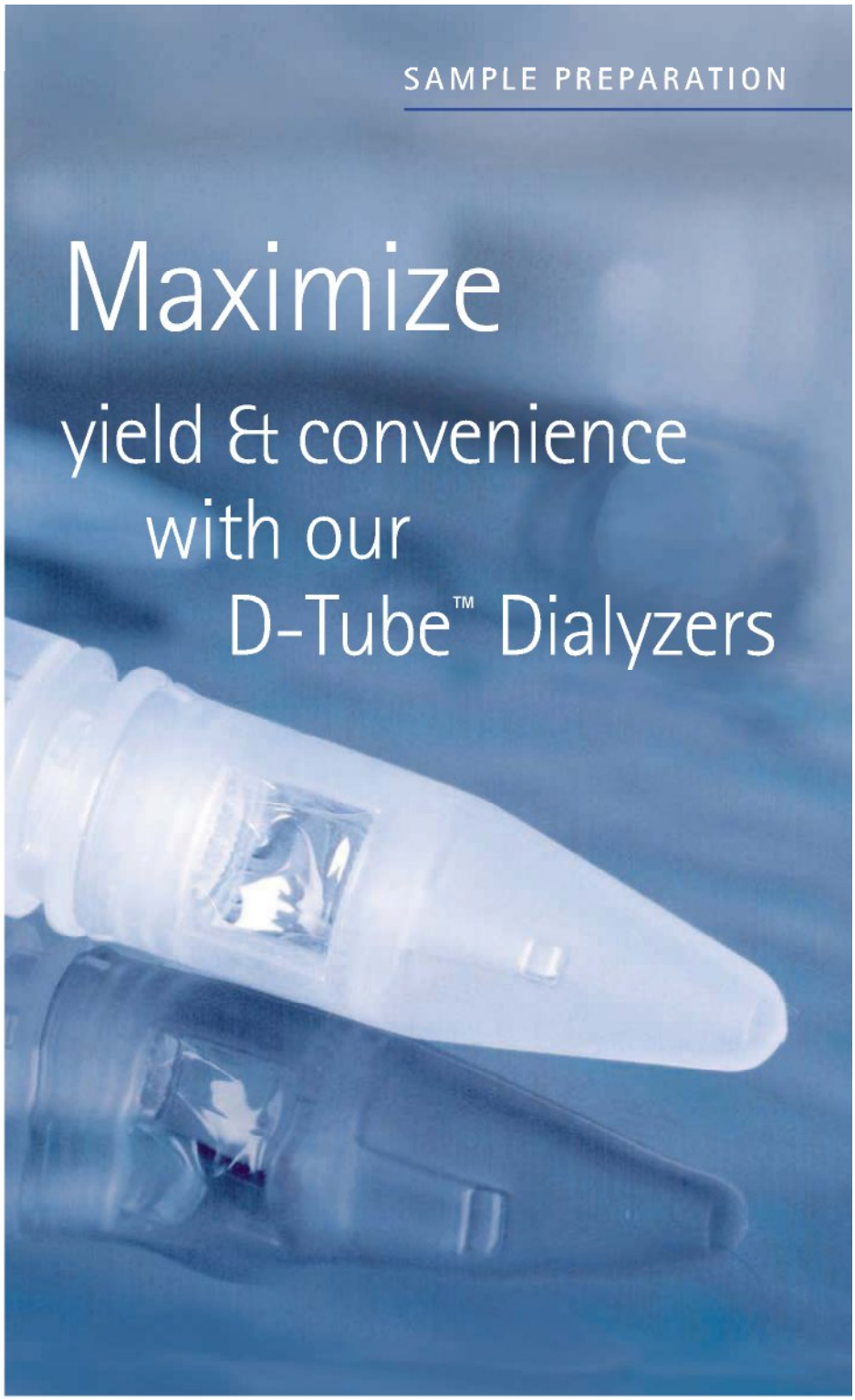

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